# Human Mesenchymal Stem Cells: The Art to Use Them in the Treatment of Previously Untreatable

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**Abstract**

Mesenchymal stem cells (MSCs) can be isolated from almost all organs and tissues in the human body. For practical purposes, there are two main sources for their isolation and ex vivo expansion – the bone marrow and fat tissue. Based on their inherent plastic adherence properties, the ex vivo expansion of

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MSCs is a rather simple process. Nevertheless, the biological features (gained from decades of tissue culture experience) are contrary to bureaucratic rules, which govern the good laboratory practice. MSCs cannot be successfully used in the treatment of human diseases if they are not handled optimally akin to the conditions in their natural habitat. Moreover, extrapolation of the data obtained from animal studies (mainly rodents) to humans is unfounded and with little relevance. The therapeutic use of genetically manipulated MSCs in human can be even harmful to patients. The current research paradigm, i.e., the use of MSCs in advanced phases of clinical trials although understandable, is far from personalized medical approach. The use of allogeneic versus autologous MSC in the clinical perspective is still debatable. There is growing evidence that the autologous MSCs derived from sick patients are "ill" in contrast to MSCs derived from healthy allogeneic donors. One can observe various changes at the DNA, RNA, and protein level in these "ill" cells. However, the huge number of cells from ex vivo expanded autologous MSCs can, possibly, overcome these aberrations. The off-the-shelf availability of allogenic MSCs also contributes to their logistic superiority over autologous cells. Moreover, due to almost non-existing immunological barriers, allogenic MSCs are emerging as gold standard and near-optimal cell types for the treatment of various diseases in humans. This chapter reviews the authors experience(s) in the treatment of various diseases with autologous/allogenic MSCs handled optimally ex vivo.

**Keywords**

Adipose tissue · Allogeneic · Autologous · Bone marrow · Ex vivo · Good biological praxis (GBP) · Human disease · MSCs · Treatment

**List of Abbreviations**

| | |
|---|---|
| DMEM-LG | Dulbecco's modified Eagle medium, low glucose |
| GMP | Good Manufacturing Practices |
| GVDH | Graft-versus-host disease |
| LG | Low glucose |
| LVAD | Left ventricular assist device |
| MSCs | Mesenchymal stem cells |
| NYHA | New York Heart Association |

## Introduction

The paper of Koç (Koç et al. 2000) symbolically "opened the door" for the mesenchymal stem cells (MSCs) in the third millennium. Here, the authors reported about the autologous blood stem cells and in tissue culture (in vitro or ex vivo) expanded bone marrow-derived MSCs in advanced breast cancer patients receiving high-dose chemotherapy. Since then, an enormous amount of material has been published (Musiał-Wysocka et al. 2019). Coming down to the molecular level, our

knowledge each day is growing exponentially. Nevertheless, the primary question remains: What is the "therapeutic mechanism" of the applied MSCs? We call this effect as "posthypnagogic command." After the treatment with MSCs, the effect of healing is present for months, without the proven presence of MSCs. We are not coming in detail here; the reader could educate himself in the enormous amount of literature.

## Mesenchymal Stem Cells Current Status: The "Problems" That in Reality Do Not Exist

### Practical Approaches: The Golden Standards, i.e., "Good Biological Praxis" (GBP) Using the Historical Laboratory Experience

In our opinion, it is useful to repeat the whole procedure of ex vivo expansion in detail as it has been described in part "*Ex Vivo* MSCs Culture" (Koç et al. 2000): "Mononuclear cells (from bone marrow) were re-suspended at $10^6$ cells/mL in Dulbecco's modified Eagle medium, low glucose (DMEM-LG) with 10% fetal bovine serum and 30 mL of cell suspension was plated in a 175 $cm^2$ flask. MSCs were cultured in humidified incubators with 5% $CO_2$ and initially allowed to adhere for 72 h, followed by media change every 3–4 days. When cultures reached more than 90% confluence, adherent cells were detached with 0.05% trypsin-EDTA." Later, additional characterizations and refinement added some regulatory rules. These ex vivo expanded MSCs fulfilled the criteria provided (later) by the International Society for Cellular Therapy (Horwitz et al. 2005). Briefly, MSCs are defined by their plastic-adherent properties under standard culture conditions, by their ability to differentiate into osteocytes, adipocytes, and chondrocytes in vitro under a specific stimulus and by positive (CD105, CD73, and CD90) or negative (CD45, CD34, CD14, and HLA-DR) expression of specific surface markers. There are two main sources for their isolation and ex vivo expansion for practical purposes – the bone marrow and the fat tissue. This ex vivo expansion of MSCs is a rather simple process based on their inherent plastic adherence properties. The pilot paper by Le Blanc et al. (2004) described the use of "third party" (here – haploidentical) MSCs for transplantation in a patient with severe treatment-resistant grade IV acute graft versus host disease (GVHD) of the gut and liver after allogeneic stem cell transplantation. For decades, two organs (or tissues), i.e., bone marrow and fatty tissue, were the primary sources for the isolation and ex vivo expansion of MSCs. The task of using allogeneic MSCs (obtained from healthy donors) or autologous MSCs in clinical settings will be discussed later.

MSCs cannot be successfully used to treat human diseases if they are not handled optimally akin to the conditions in their natural habitat. Let us discuss this in depth. As an example, we will consider the research paper published by Yau and colleagues (Yau et al. 2019). The authors claimed that "among patients with advanced heart failure, intramyocardial injection of mesenchymal precursor cells, as compared with the injections of a cryoprotective medium as sham treatment, did not improve successful temporary weaning from left ventricular assist device (LVAD) support

at 6 months. These findings do not support the use of intramyocardial MSCs to promote cardiac recovery as measured by temporary weaning from device support." According to the authors, the patients were randomly assigned to cell therapy group who received intramyocardial injection of 150 million MSCs and a cryoprotective medium treatment group without cells for comparison. The allogeneic MSCs were obtained from healthy donors and expanded in a Good Manufacturing Practices (GMP) certified laboratory. It is evident that the cells were thawed directly before use ("injections of mesenchymal precursor cells, compared to injections of a cryoprotective medium as sham treatment"). The cells were neither washed nor cultivated further for expansion before use. In the opinion of the authors that it is mandatory to use the MSCs that have been freshly prepared and not frozen or thawed immediately before use.

After decades of expanding the MSCs (and other cells) ex vivo (in vitro), we firmly stand behind this point of view. After thawing, the cells need to be cultured at least for 48 h in humidified incubators supplemented with 5% $CO_2$. Only after this wait period, one should start to consider further experimental (or therapeutic) work using these cells. On the other hand, one can consider growing the cells ex vivo, detaching them when 80% confluent and applying them to the patient in a short time (up to 3 h at room temperature). The practice to use freshly thawed cells (MSCs) makes the abovementioned study (and others in this fashion designed trials) from the biological point of view rather dubious and medically useless.

It should be noted that the number of skeptical articles and comments about the relevance of the MSCs for cell-based therapy is growing (Gomez-Salazar et al. 2020; Curfman 2019). We strongly disagree with the emerging notion. In our opinion, it is necessary to return to the laboratory and to give the MSCs a "second chance" by consequently following the GBP developed during the decades of cell tissue culturing in vitro. We recommend returning to the praxis of small tissue culture centers associated with (or localized within) the hospitals. In coordination with the hospital departments, they could prepare fresh MSCs, which would be "on demand" prepared for use and treat the patients. Logistically, to prepare a total of 20–50 $\times$ $10^6$ cells is not a difficult task. One skilled technician could obtain this amount under sterile conditions in 1–2 h. What about the tests for the differentiation and of sterility? Well, yes, one can ask a heretical, unorthodox question: Did anybody ever observe that the MSCs in vitro did not differentiate to osteoclasts, adipocytes, and chondrocytes during appropriate treatment? This has been further discussed elsewhere in the chapter.

## Data Extrapolation from Small Animal Studies to Humans Is Unfounded and Without Relevance

The therapeutic use of genetically manipulated MSCs in humans is even harmful to patients. The engineered ("therapeutic") MSCs are genetically modified MSCs that contain a stable gene encoding for protein or enzyme product/s able to kill the tumor cells. A typical example of such a construct is the yeast enzyme cytosine deaminase,

which converts the rather nontoxic 5-fluorocytosine to the cytostatic agent 5-fluorouracil (Kucerova et al. 2007). The results obtained from rodents, i.e., preclinical experimental models, are promising. In a recently published review article, Pawitan and colleagues have stated: "So far, most studies using pre-clinical cancer models have shown consistent results, i.e., the engineered MSCs could inhibit tumor growth and enhance the survival rate of the tumor-bearing animals" (Pawitan et al. 2020). And only one published clinical paper by Lakota et al. (2015) claims the opposite: "Treatment with therapeutic MSCs (i.e., genetically engineered MSCs) of this patient highlighted the following points" (Table 1):

1. There was no evidence of any therapeutic benefit after intravenous administration (not local, i.e., intra-tumoral injection) of the therapeutic MSCs. Six days after the cell administration, the metastatic process did not show any signs of regression. Moreover, 40 days after the treatment, there was a progression of the metastases.
2. After the intravenous administration, the therapeutic MSCs were probably "homing" into the bone marrow despite their adipose tissue of origin. Even a relatively low cell count ($60x10^6$) was able to cause grade 2 (resp. grade 3) thromobocytopenia (resp. neutropenia)

It should be noted that this patient did not receive any systemic chemotherapy in the past. The observed bicytopenia with a nadir neutropenia occurred 48 h after administering therapeutic MSCs (with concomitant prodrug administration). Moreover, in a more recently published paper by Lakota (2018), the author claims that:

(i) There was no sign of any therapeutic effect after intravenous administration (not local, i.e., intra-tumoral) of the "therapeutic" MSCs.
(ii) After the intravenous administration, the "therapeutic" MSCs were "homing" into the bone marrow.
(iii) There has not been any entrapment of the "therapeutic" MSCs in the lungs (after the intravenous administration).

**Table 1** Blood counts of the patient with head and neck tumor during and after the therapy with therapeutic MSCs. (The table is taken from Lakota et al. 2015)

| Day | Leukocytes ($\times10^{-12}$/l) | Neutrophils ($\times10^{-12}$/l) | Erythrocytes ($\times10^{-15}$/l) | Hb (g/l) | Plt ($\times10^{-14}$/l) |
|---|---|---|---|---|---|
| −2 | 6.88 | 4.86 | 4.41 | 134 | 179 |
| 0 | 5.82 | 3.98 | 3.71 | 119 | 150 |
| +1 | 4.06 | 3.41 | 3.80 | 119 | 119 |
| +2 | 1.99 | 1.00 | 3.41 | 112 | 100 |
| +3 | 2.89 | 1.58 | 3.72 | 117 | 115 |
| +4 | 3.50 | 2.16 | 3.72 | 115 | 122 |
| +5 | 4.50 | 2.98 | 3.79 | 118 | 129 |
| +6 | 4.29 | 2.63 | 3.89 | 121 | 132 |
| +18 | 6.57 | 4.66 | 4.06 | 125 | 191 |

(iv) After local (i.e., in situ) administration, the "therapeutic" MSCs did not migrate to any "neighboring" tissue/organ (the liver, retroperitoneum, abdominal wall) or other distant organs, including the bone marrow.

Thus, the data obtained from small experimental animal models (mice and rats) must not be extrapolated to the humans. Moreover, the unrestrained transfer (although understandable) of the requisitioned data obtained with the MSCs from rodents may negatively influence the research trends in the novel treatment(s) of human diseases. This kind of treatment of human patients is ineffective and can be harmful and dangerous. The systemically administered therapeutic MSCs are rather homing into the patients' bone marrow and not into the tumor tissues. After local application (in the tumor area), their ability to destroy the whole tumor or its metastasis is limited. Nor they are moving to other metastatic tumor localizations.

## Current Research Paradigm: MSCs in Advanced Phases of Clinical Trials Although Understandable Is Far from Personalized Medicine

Current praxis is treating human diseases based on evidence-based medicine (Masic et al. 2008, and the Internet). Clinical trials are the cornerstone to assess novel disease treatment(s), and among the clinical trials, randomized clinical trials are considered as the most preferred design as it provides a causal relationship between the medical intervention and the desired effects (Sawchik et al. 2018). A pertinent question here is: Is the same approach valid for MSCs which is fast emerging as a biopharmaceutical? Here we present some of our (published and unpublished) data none of which has been acquired in any registered trial. All of these data were obtained according to the rules of personalized medicine.

(i) Autologous bone marrow-derived MSCs were used for transplantation in ten patients with ischemic cardiomyopathy (Lakota 2014a). All patients had a well-documented history of anterior wall acute myocardial infarction. They were in NYHA stage III or IV. The freshly prepared MSCs were injected into the left anterior descending coronary artery. All of the patients tolerated the MSCs injection well and were discharged from the hospital 3–4 days after the procedure. Six patients died 7, 9, 37, 71, 101, and 119 months after the procedure. Four patients were alive 112, 113, 120, and 121 months after the MSCs-based treatment.

(ii) The allogeneic adipose tissue-derived MSCs were used for the treatment of the patient with 11 years history of ulcus cruris on his left leg, which remained stationary despite trials of conservative treatment (Lakota 2014b). The adipose tissue-derived MSCs ($30 \times 10^6$) were applied locally (circumferentially). After a single round of MSCs treatment, the ulcus healing progressed "normally" despite repeated courses of standard and high-dose chemotherapy for patient's oncological diagnosis (high-grade non-Hodgkin's lymphoma). The final effect, i.e., restitutio ad integrum, has been observed during 6 months after

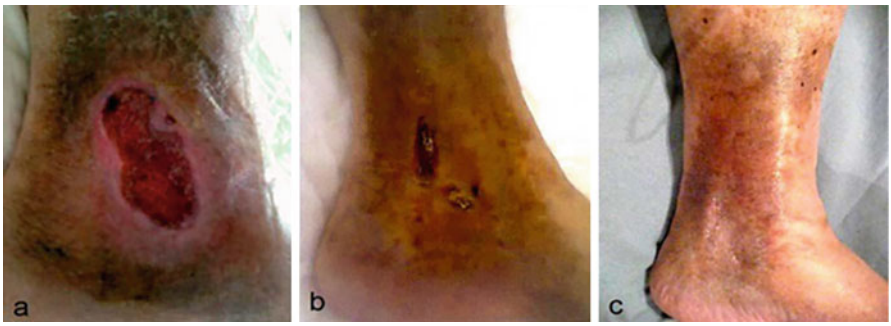


**Fig. 1** Healing of the ulcus cruris after local application of MSCs. (**a**) 11/2012, day after application; (**b**) 02/2013, d + 25 after autologous stem cell transplantation; and (**c**) 05/2013. (The figure is taken from Lakota (2014b))

the MSCs-based treatment (Fig. 1). This course of healing did not differ from the patients with ulcus cruris who received allogeneic or autologous MSCs and who did not receive any chemotherapy (Lakota, unpublished).

(iii) Ten patients after allogeneic stem cell transplantation (high-dose therapy for hematological malignant disease) and with severe steroid-resistant grade IV acute GvHD were treated with allogeneic bone marrow-derived MSCs (range 0.3–0.5 $\times$ $10^6$ MSCs/kg body weight) (Lakota 2017a). GvHD was successfully resolved in four patients who received MSCs-based therapy (Table 2). In the end, the author claimed: "Moreover, it seems plausible to have the MSCs 'in stock' for fast *ex vivo* expansion to use the freshly prepared cells (rather than frozen, thawed, and immediately used)."

(iv) The autologous bone marrow-derived MSCs and allogeneic adipose tissue-derived MSCs were used to treat aseptic necrosis of the jaw. The treatment was performed in 30 patients over a period of 15 years. Freshly prepared MSCs ($20 \times 10^6$) from the respective tissues were applied locally. In five patients, MSCs were used twice. The result was a restitutio ad integrum (Fig. 2) (Lakota, unpublished data and Lakota 2017b, in Slovak).

(v) The allogeneic adipose tissue-derived MSCs were used for the treatment of ten patients with sclerosis multiplex. The target dose has been 0.5 $\times$ $10^6$ cells/kg bodyweight. The patients were advised not to stop current "official" treatment. Freshly prepared MSCs were delivered intravenously. No adverse effects were observed. The CNS lesions in all patients but one did not progress during 15 years of follow-up after MSCs-based cell therapy (Lakota, unpublished data).

We did not mention all the data obtained by the authors during the last two decades involving MSCs-based cell therapy. The authors never performed any double-blind randomized clinical trials due to logistic and economic reasons. In our hands, personalized medicine and the medical treatment followed the golden rule: "primum non nocere." We followed the aim and principle of helping the sick

**Table 2** Patients' characteristics, date of transplantation, date of MSCs infusion, date of the patients' death, and the reason of death. (Haplo Tx: HLA haploidentical transplantation; RI Tx: reduced intensity conditioning transplantation). (The table is taken from Lakota 2017a)

| Patient No | Gender | Born | Diagnosis | Transplantation | MSC infusion | Death | Reason of death | Comment |
|---|---|---|---|---|---|---|---|---|
| 1 | M | 1971 | AML | 16.03.04 | d + 206 | 09.11.04 | cGvHD | |
| 2 | M | 1976 | HD | 30.01.05 | d + 1 | 14.02.05 | aGvHD | Haplo Tx |
| 3 | M | 1971 | ALL | 13.12.05 | d + 9 | 05.04.06 | Disease progression | |
| 4 | F | 1970 | Ph-CML | 26.04.07 | d + 33 | – | Alive (31.12.16) | |
| 5 | F | 1978 | HD | 13.10.09 | d + 60, d + 80 | 23.02.10 | aGvHD | RI Tx |
| 6 | M | 1969 | ALL | 23.02.10 | d + 168 | 16.08.10 | cGvHD | |
| 7 | F | 1973 | HD | 21.10.10 | d + 92, d + 178 | 22.09.11 | Infection | HaploTx |
| 8 | M | 1987 | HD | 18.10.11 | d + 43 | 09.04.13 | Disease progression | RI Tx |

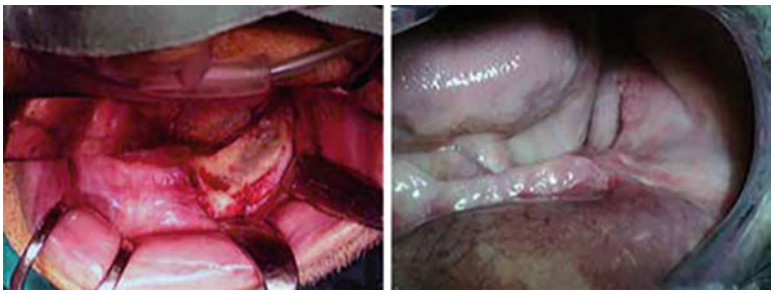

**Fig. 2** Healing of the osteonecrosis of the jaw after local application of MSCs. Left: during the surgery; right: 12 weeks after the surgery. (The figure is taken from Lakota 2017b)

and suffering patients in concert to see how serpentine can be the road to establish a new treatment in medicine. It is worth mentioning the following observation. In 2004, Vulliet and colleagues published an interesting research paper of using MSCs in a healthy dog (Vulliet et al. 2004). The authors delivered 0.5 million MSCs/kg bodyweight into the canine left circumference coronary artery. The authors observed ECG changes (ST-segment elevation) in all dogs receiving cell administration which was a characteristic of acute myocardial ischemia. Besides microscopic and macroscopic evidence of myocardial ischemia, the authors also observed increased troponin I in two dogs in which measurements were made. These data suggested that myocardial ischemia occurred after the injection of MSCs at the dose used in this study. Microinfarction was also confirmed with histological and immunocytochemical data.

These results showed a potential complication of injecting MSCs, or probably any similarly sized cell, into the coronary circulation. Although differences between canine and human coronary circulation exist, and different cell types and sizes have been used for selected cytotherapeutic applications, this potential complication should be thoroughly investigated before MSCs are routinely injected into the arterial circulation of the patients. Luckily for us (Lakota 2014a) and for the patients at the time of publishing this paper (Vulliet et al. 2004) as we became aware of that we performed three intracoronary MSCs transplants and three other patients were "on the horizon." Thus, this is another confirmation of how dubious the translation of "(pseudo)clinical" data obtained on animals to human clinics is. Two recently published reviews in this regard have elegantly discussed the donor-related factors and quality of the cell preparation as the possible determinants of the outcome of cell-based therapy in the clinics (Haider 2018; Rady et al. 2020).

## A Case of Autologous Versus Allogeneic MSCs, an Unanswered Question

One of the ongoing and inconclusive debates in cell-based therapy is the preference for autologous over allogeneic MSCs. However, the use of allogeneic MSCs for

cell-based therapy is fast emerging as a new paradigm in therapeutics (Karantalis et al. 2016). Starting with the pioneering work of Orlic et al. (2001), preclinical studies have characterized syngeneic, xenogenic, allogeneic, and autologous MSCs; however, most of these studies, especially in the small animals, i.e., mice, rats, etc., have focused on the use of allogenic MSCs due to ease of availability (Orlic et al. 2001, Fukuda and Fujita 2005; Jiang et al. 2006; Haider et al. 2008; Beitnes et al. 2012). Similarly, in large animal translational studies, autologous and allogeneic MSCs have been used without any safety issues with either cell type (Poh et al. 2007; Quevedoa et al. 2009; Chen et al. 2014). But then the question remains why these data have been ignored while designing the clinical trials wherein mostly autologous MSCs have been focused on cell-based studies.

As evidenced by a systematic review and meta-analysis of 82 animal studies involving 1482 animals that both autologous and allogeneic cells are equally effective (Jansen of Lorkeers et al. 2015), but they have their respective advantages and limitations in the clinical perspective. Hence, the clinical researchers should give these parameters due consideration during clinical study design for optimal therapeutic outcomes. The following section discusses in depth the pros and cons of each cell source in the light of the published data and implores that the relevant information should be given due consideration, especially during the design of clinical studies.

## A Case for Autologous Cells

On its face, the fear of incompatibility is alleviated with the use of autologous cells. Hence, autologous cells' usage is advantageous as it does not require immunosuppression to support the acceptance of the transplanted cells and their derivative tissue after differentiation posttransplantation. Moreover, treatment with autologous cells is considered a step closer to the fast-emerging personalized medicine. However, on the downside of autologous cell-based therapy, the use of autologous cells is time-consuming and labor-intensive exercise, and clinically less viable option, as it may necessitate biobanking of autologous cells for future use of the cells for every individual. On the same note, it is also not sustainable for diseases where the patient may require early intervention, i.e., myocardial infarction, stroke, etc., and the patient does not have the choice of long waiting time until the harvested cells can be purified and expanded to achieve the required cell number for transplantation. Some essential considerations in case autologous cells are as follows:

### Autologous Cells from Patients Are Also "sick"

There is growing evidence that the autologous cells derived from the patients eligible for the cell-based treatment are "sick" compared to the ones derived from healthy allogeneic donors due to their exposure to different risk factors and comorbidities in the "sick" donors (Dimmeler and Leri 2008; Cesselli et al. 2011). Moreover, it is now well-documented that many diseases compromise the stem cell niche homeostasis and seriously affect stem cell properties such that they are rendered unsuitable for cell-based therapy (Perez et al. 2018). For example, Liu et al. have shown that MSCs from diabetic patients have impaired cardioprotective function as compared to the

ones derived from the healthy donors, which was ascribed to the long-term exposure to hyperglycemia (Liu et al. 2013).

We have performed RNA microarray analysis comparing MSCs from two patients with ischemic cardiomyopathy and two healthy donors (Lakota 2014a). Data analyses showed a significantly enhanced gene expression ratio for STAT1α/β (signal transducer and activator of transcription 1-alpha/beta) and ISG15 (ISG15 or g1p2 or ucrp; ubiquitin cross-reactive protein). The decreased ratio of gene expression has been shown for GTP-binding protein RAD. The first analyzed patient died 101 months after the procedure. The second one was alive 120+ months after the procedure.

It should be noted that the MSCs in all patients treated were isolated from their bone marrow. This data clearly shows that ischemic cardiomyopathy is a "systemic" disease. One can speculate about the following fact: How is it possible that the patients with this "damaged" RNA profile in MSCs survived such a long time after the procedure, i.e., such "sick" MSCs were able to repair the damaged myocardium?

### Autologous Cells in Elderly Patients Are Also Aged

Another critical aspect generally overlooked during the use of autologous cells is the donor age besides the age of the recipient. A considerable majority of the patient population who are candidates for cell-based therapy are elderly. Using their own (autologous) cells for cell-based therapy and transplanting them back into an aging tissue environment (which has lost the vigor of reparability due to chronological aging) accounts to double negative that significantly hampers the therapeutic outcome (Zhuo et al. 2010).

It is pertinent to mention that similar to any other body cell, stem cells also undergo chronological aging, which is multifactorial, i.e., metabolic alteration, accumulation of reactive oxygen species, accumulation of DNA damage and mutations, telomere shortening, etc. (Oh et al. 2014; Schultz and Sinclair 2016). These metabolic and molecular-level changes lead to loss of their stemness characteristics with age, which is reflected in the impairment of their functionality (Kissel et al. 2007; Bustos et al. 2014). Moreover, chronological aging also leads to a significant reduction in stem cells (Maijenburg et al. 2012). Stenderup et al. carried out a direct in vitro comparison of bone marrow-derived MSCs from young donors (18–29-year-old, $n = 6$) versus elderly donors (68–81-year-old, $n = 5$) (Stenderup et al. 2003). The authors compared the cells for the expression of senescence markers, cell growth, and differentiation potential. It was observed that the cells from elderly donors showed significantly reduced maximal life span in terms of population doublings (PD) and PD rate, and accelerated senescence as evidenced by beta-galactosidase expression as a marker. Similarly, a comparison of bone marrow-derived MSCs from human donors (17–90 years age) showed a significant increase in doubling time beside increased expression of senescence markers with the advancing age of the donor (Zhou et al. 2008).

Chronological aging and obesity also cause a decline in stem cell yield and their ability to hematopoiesis and bone regeneration (Pachon-Pena et al. 2016, Ambrosi et al. 2017). Therefore, it is essential that clinical researchers consider the

consequence of donor age while opting for autologous cell sources (Stolzinga et al. 2008). A direct comparison of the reparability of bone marrow-derived MSCs showed that the rate of old donor bone marrow-derived MSCs had poor cardiomyogenic differentiation potential as compared to the MSCs derived from young donor bone marrow (Jiang et al. 2008). For comparison, the cells were transplanted in the same heart in an experimental animal model of acute myocardial infarction. Khan et al. showed that the reparability of the senescent myocardium is determined by the age of the donor (Khan et al. 2011). All these data signify that MSCs from aging patients show a drastic loss of their biological activity and bring us to an important question if the use of autologous is the real culprit for the modest outcome of the clinical trials reported to date (Shahid et al. 2016).

## A Case for Allogeneic Cells

Data emanating from the clinical studies have shown the safety and efficacy of allogeneic MSCs in adult and pediatric patients (Koc et al. 2002; Horwitz et al. 2002). Recent clinical application of allogeneic MSCs in ischemic cardiomyopathy patients vindicated these data and reported that allogeneic MSCs were as good as autologous MSCs in their functionality and efficacy, favorably affecting LV end-diastolic volumes, LVEF, and ventricular remodeling leading to improved quality of life (Hare et al. 2012, 2017). More importantly, these studies did not report any severe adverse reactions associated with the cell-based therapy with allogeneic cells, including immunologic responses. The safety profile of allogenic MSCs has also been substantiated during a systematic review and meta-analysis of 36 clinical studies, including 1012 participants (Lalu et al. 2012). Experimental studies assessing immunological profiling of MSCs have shown that although they are not immunopriviledged, allogeneic MSCs are weakly immunogenic, because they lack MHC class II and co-stimulatory molecules, i.e., CD40, CD80, and CD86, while they have weak MHC class I expression (Machado et al. 2013; Lohan et al. 2014). Moreover, they do show immunomodulation by suppressing the activation and proliferation of immune cells (Asari et al. 2009; Corcione et al. 2006). Their interesting immune profile tips them as a good candidate for cell-based therapy without the need for immunosuppression therapy and takes care of them not being "self" for the recipient (Kariminekoo et al. 2016). These data about the allogeneic MSCs are a step forward towards the ongoing quest for "Universal donor cells," which should be available off-the-shelf as a ready-to-use cell preparation (Kinkaid et al. 2010).

### Logistic Advantage of Allogenic MSCs

One of the primary advantages of allogenic MSCs is their logistic superiority over autologous cells (Zhang et al. 2015). Unlike autologous MSCs, which need to be isolated, purified, and expanded in culture before use for each patient, allogenic cells are logistically feasible as they may be readily available off-the-shelf. This ready availability makes possible their use in urgent clinical situations, which is not possible with the autologous cells as it may take 3–4 weeks of isolation, purification,

and expansion before they could be used for delivery. For any cell-based therapy to be of routine clinical significance as a therapeutic modality, it is imperative that the cells must be available off-the-shelf akin to any other conventional pharmacological agent. Despite the fast-emerging innovative field of personalized medicine, drugs are not to be synthesized for each patient; instead, their use is tailored according to the need of the patients who are stratified to enhance therapeutic efficacy (Marshall et al. 2016).

Similarly, in cell-based therapy, which is one form of personalized medicine, cells cannot be prepared for each patient before use; there have to be readily available cell preparations, which can be tailored to the need of the patient. Manufacturing large clinical grade batches of allogenic cell products using GMP, quality controlled for viability, self-renewal, and stemness characteristics will be cost-effective, time-saving, less labor-intensive, commercially favorable, and clinically more relevant for reproducible outcome. Moreover, this off-the-shelf approach fits well with the current pharmaceutical practices, scale-up manufacturing, and may involve automation to make it efficient in manufacturing, thus having a better commercial potential than autologous cells (Malik and Durdy 2015). Additionally, allogeneic MSCs may allow repeated doses of the cells which may be more beneficial than one-time treatment (Poh et al. 2007).

### Allogenic MSCs Overcome the Limitations of Aging and Sickness of Autologous Cells

As discussed earlier, autologous cells derived from elderly patients, especially those with multiple comorbidities, may not fetch the desired results. Availability of allogeneic cells from a young healthy may be a better option for cell-based therapy. In a recently published study, which was aimed to identify a set of donors and their donated cells' characteristics with predictive value for optimal osteoblastic differentiation, bone marrow-derived MSCs from 58 patients undergoing surgery for bone fracture were characterized for high osteogenic potential and low adipogenic potential (Kowal et al. 2021). The authors have reported a well-defined criterion that donor cells obtained from male donors, without a diagnosis of osteoporosis and containing a higher fraction of $CD146^{+}$ fraction of cells, together were predictors of high osteogenic potential. Such predefined criterion is also warranted for selecting MSCs for use in the patients who are candidates for cell-based therapy for other diseases.

We will not go into detail here (Lakota 2016); nevertheless, one possible explanation could be that the myocardial repair occurred because of the presence of a considerable amount of ex vivo expanded MSCs. The RNA microarray analysis reflects only the statistically up- or downregulation of specific genes. In another study, Koh and coworkers suggested that pluripotency and the secretion of trophic factors of the bone marrow-derived MSCs in amyotrophic lateral sclerosis patients were reduced in proportion to a poorer prognosis. This may suggest that allogeneic (bone marrow- or adipose tissue-derived) MSCs from healthy donors may be a better option for MSCs therapy in amyotrophic lateral sclerosis patients (Koh et al. 2012).

## Quality Control of MSCs Preparation: A Prerequisite for Optimal Prognosis

Autologous or allogeneic MSCs are part of the primary prerequisite of quality of the MSCs preparation for use in the patients (Haider 2018) and remain a fundamental determinant of the outcome of any cell-based therapy procedure and its success (Haider 2017). It encompasses everything from percentage cell count and cell viability, their identification to proliferation and differentiation potential to paracrine action of the cells in the MSCs preparation to ensure that the cell preparation is the best compromise of all these properties. For example, the cells should have the proliferation capacity, but at the same time, unlimited proliferation will add the risk of tumorigenicity. Hence, validating MSCs preparation, both biologically and functionally, will ensure that the cells will do the needful, which is meant for post-engraftment in the clinical settings. Unfortunately, these critical aspects of quality control of the cell preparation in general, and functional assessment in particular, have been generally overlooked during the design of the clinical studies. This has seriously impacted the efficacy of the cells, thus significantly contributing to the modest outcome of the clinical trials in most cases.

For example, bone marrow-derived MSCs, both autologous and allogenic, require in vitro culturing for at least 3–4 weeks. Out from their natural habitat, they are exposed for so long to the unnatural biological environment, which is only partially emulating their natural habitat at best, is expected to alter their biological as well as functional characteristics significantly. All this leads to their senescence or aging in culture due to less than optimal culture conditions (Bonab et al. 2006; Jiang et al. 2017; Shen et al. 2018). Moreover, the long-term culture may render the cells devoid of their specific surface marker expression, i.e., CD29, CD44, CD90, and induce chromosomal instability (Furlani et al. 2009). Transplantation of these in vitro expansion showed no functional effect post-engraftment in experimentally infarcted myocardium. Although various strategies have been developed to recover the culture-induced senescence of cells in terms of their proliferation capacity (Koichi et al. 2011; Tan et al. 2021), the cells may become transformed to be tumorigenic depending upon the culture condition, thus becoming unsafe for cell-based therapy (Rosland et al. 2009; Wolf et al. 2009).

Similarly, the long-term culture of the cells becomes immunogenic due to the settlement of extraneous proteins from the culture medium. These ill effects of prolonged in vitro culture are most pronounced in 2D culture conditions. It is anticipated that the advanced 3D culture conditions in vitro will go a long way in alleviating the effects of cell culture-induced cell senescence due to its biomimetic properties (Hoch and Leach 2014; Jeger-Madiot et al. 2021).

Another typical example is the effect of cryopreservation of the cells in clinical settings. Although the current cryopreservation protocols are well-optimized and successfully preserve the biological and functional characteristics of the cryopreserved cells, some aspects of the technique require further refinement (Mamidi et al. 2012). Cryopreserved MSCs show altered immunomodulatory and therapeutic efficacy with a significant reduction in the number of viable cells. The rate of cell viability, as well as cell clumping, is also an important complication of

cryopreservation that may contribute to micro-occlusions after intra-arterial delivery of the cells as compared to the freshly isolated cells with cryopreservation (Cui et al. 2016). A recent study has reported that the cryopreserved successfully maintained their multi-lineage differentiation, immunomodulatory and anti-inflammatory properties, but they lose some of their stemness characteristics in a reversible fashion, which the cells could recover within 24 h in the culture after thawing (Antebi et al. 2019). Kaplan et al. have elegantly reviewed the effect of cryopreservation of the MSCs biological and functional characteristics (Kaplan et al. 2017).

## Conclusion

In our opinion, the question of the use of autologous versus allogeneic MSCs remains unresolved. Autologous MSCs, although sick, are returning home after ex vivo expansion when they are used for cell-based therapy. Allogeneic healthy cells are here on demand. The immunological barriers do not (in practice) exist. According to the authors' experience, one should not be afraid to use them in all cases when there are doubts about the current availability of autologous MSCs. No single negative effect has been ever observed. Therefore, the summary of theoretical pros and cons cannot solve up to date this problem.

In conclusion, we would like to remind the reader of the old rule which governs the laboratory praxis. This is what we call "good biological praxis" (GBP). One should not create problems where they do not exist. Or, better, with humbleness, we should approach the divine principles in nature which we receive as gifts. With these gifts earlier hidden, we will be able to treat previously untreatable. MSCs are the current example. Human medicine is hotly waiting.

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