Chapter 19
Mesenchymal Stromal Cells in the Clinic: What Do the Clinical Trials Say?

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Abstract In the last 5 years, a large number of trials have been undertaken for the clinical application of cell-based therapy with human mesenchymal stromal cells (MSCs) for a variety of human autoimmune and degenerative diseases. In preclinical and clinical studies with ischemic injury, diabetes, wound healing, graft versus host disease, MSCs are emerging as promising candidates with therapeutic potential. MSC features such as homing efficiency to injured site, ability to produce several trophic factors in critical quantities needed for repair, immunomodulatory features to facilitate engraftment are expected to be the underlying mechanisms for therapeutic benefit in these disease states. Although early results are promising, much work is required as cellular therapies need careful isolation of cells, expansion, characterization, and proper delivery of injectable transplant ready cells that need to be prepared in good manufacturing practice (GMP) conditions to meet the safety and specification, reproducibility with no or minimal lot-to-lot variation, and efficacy following transplantation in to disease subjects. There have been 230 clinical trials as of April 2012 with MSCs that have been registered with Food and Drug Administration (FDA) site in various stages of investigation with autologus as well as allogenic sources of bone marrow-derived cells. This review summarizes the outcome of the completed trials and lays foundation for the expected outcome of the ongoing trials.

Keywords MSC • Clinical trials • Preclinical trials • Cardiac • Stroke • Spinal cord injury • Bone and cartilage • Diabetes • Critical limb ischemia • GVHD

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19.1 Introduction

Mesenchymal stromal cells (MSCs) are multipotent cells, found in a variety of tissues like bone marrow, adipose tissue, dental pulp, skin, etc. These cells can differentiate into tissues of mesodermal origin like bone, cartilage, fat, skeletal muscle, etc. In addition MSCs have also shown to be able to differentiate to tissues of ectodermal origin like neurons. This ability of MSCs to differentiate into a variety of tissues makes them attractive therapeutic agents to treat a variety of disorders. This review will focus on summarizing the ongoing and recently completed clinical trials using MSCs. This review does not attempt to summarize the status of MSCs in the clinic, as excellent reviews on that topic [1, 2] are recently published.

MSCs are easy to culture and unlike hematopoietic stem cells, can be expanded in culture without differentiation. These cells can also be grown in defined xeno-free media for therapeutic use. The cultured cells can be characterized by the use of surface markers such as those designated by the International Society for Cellular Therapy (ISCT) [3]. Although many clinical trials use undifferentiated MSCs, these cells can also be differentiated into specific lineages in vitro. Further these cells can also be grown on numerous scaffolds which could be used for tissue engineering.

MSCs are already being used in a number of clinical trials for treating variety of disorders. A search on the NIH clinical trials.gov database yielded over 230 trials utilizing MSCs for treating a variety of disorders (Fig. 19.1). Of these 40 trials were completed while an equal number had stopped recruiting, but were still active. Three trials were suspended, or withdrawn and one trial for heart failure was terminated. The remaining trials were still actively recruiting, highlighting the enormous interest in this area. Thirty-seven trials were phase I trials, 90 were intermediate between phase I and phase II, 53 were phase II and 11 were phase III studies (Fig. 19.2).

In these trials, patient enrolment numbers varied from 8 to 10 patients for small studies to 290 patients in a phase II study of osteoporotic fractures. Majority of these trials were interventional while only 9 trials were observational. Forty-five of these trials were funded purely by industry, 15 by a combination of industry and academic research institutions while the remaining were investigator lead. This shows that majority of trials involving MSCs are being led by investigators and very few are being lead by companies which desire to launch a product. One hundred and three of these trials were focused on or involved children. This highlights the importance of MSCs in early intervention of childhood disorders that can be manifested at a later age.

These trials involved the use of MSCs in a variety of disorders. Liver failure was the largest indication (19 studies), followed by graft versus host disease (14 studies). Osteoarthritis, critical limb ischemia, Crohn's disease, type 1 and type 2 diabetes were the other disorders involving the use of MSCs for clinical trials.

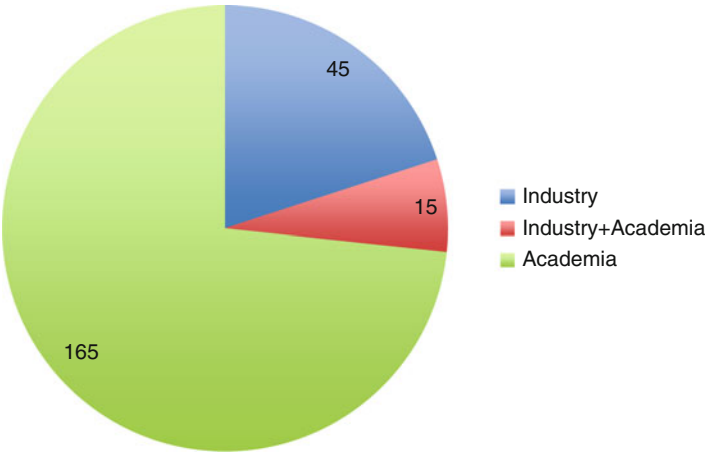

Fig. 19.1 Distribution of MSC trials funded by Academia and Industry. The pie chart indicates proportion of MSC trials funded by Industry vs. Academia. The figure shows that while majority of the trials are Academia funded, a significant proportion of clinical trials are funded by Industry. This highlights the commercial interest in the use of MSC as therapeutics. The *numbers* indicate number of trials

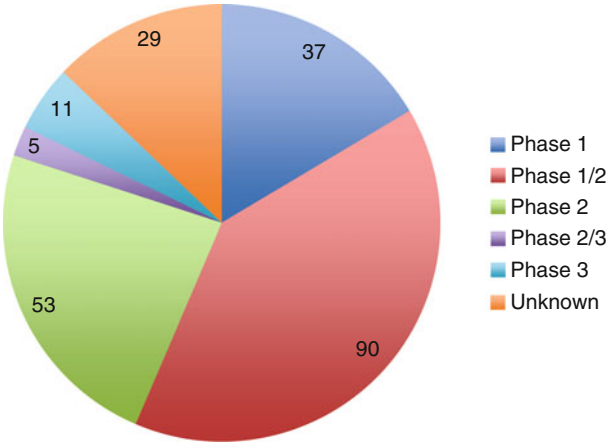

Fig. 19.2 Distribution of MSC trials dependent on their phase of study. The figure shows that majority of the trials are in phase I or intermediate between phase I and phase II. Very few trials have reached phase III. This is reflective of the nascent nature of this field since most trials are early trials to determine primarily safety and in some cases efficacy of transplanted MSCs in treating these disorders. The *numbers* on the pie chart indicate number of trials

19.2 MSCs for Cardiac Therapy

There was tremendous excitement in the field for use of MSCs to improve cardiac function. The initial trials like the SCOPE study were predominantly safety studies assessing the safety of bone marrow collection or MSC administration in patients with acute myocardial infarction or congestive heart failure. After the initial euphoria of using MSCs in acute myocardial infarction as well as congestive heart failure, it was clear that transplanting these cells had limited beneficial effect. Recent evidence indicates that MSCs do not form cardiomyocytes, but may have indirect beneficial effect on cardiac function [4, 5]. Although initial small studies reported benefits of MSC transplantation, these beneficial effects were not observed in larger controlled randomized trials. The clinicaltrials.gov database lists 11 current or completed trials involving the use of MSCs in treating cardiac disorders. Of these 3 trials were carried out for myocardial infarction, whereas one trial at the Rigshospitalet in Denmark is exploring the use of MSCs for treating congestive heart failure. All these trials involve the use of bone marrow MSCs. One trial in Mexico is using autologous adipose-derived stem cells to treat heart failure. Only one trial by US-based Capricor will be using allogenic trials for acute myocardial infarction. A recently completed STEMI trial in India also used allogenic MSCs for myocardial infarction (CTRI/2009/091/000176).

Many of the beneficial effects of MSCs can be attributed to indirect effects. Transplanted MSCs can contribute to neoangiogenesis in cardiac tissue. Exosomes secreted by MSCs have also been shown to improve cardiac function [6]. Lu et al. reported that macrophages in damaged myocardium phagocytose transplanted MSCs and secrete factors that stimulate stunned myocardium [7].

Thus it is possible that the beneficial effects of MSCs in treating cardiac disorders do not actually result from direct transplantation of MSCs. Therefore it may be possible to create MSC-based therapeutics for treating cardiac disorders. This may obviate the need for direct transplantation of MSCs since MSC-derived products like cytokines, exosomes or cellular fragments may be sufficient for these treatments. Thus the most important message from all these clinical trials is the possibility that the endogenous stem and progenitor cells have the potential to participate in repairing and restoring the diseased cardiac function. These insights although optimistic underline the critical need for better designed clinical trials with clear end point read outs and better prediction of efficacy of cell therapy.

19.3 MSCs in Neurological Disorders

Although MSCs can differentiate into neurons with low efficiency, this has not stopped the investigational use of MSCs for treating neurological disorders. The clinicaltrials.gov database lists 19 trials utilizing MSCs to treat neurological disorders. These trials cover a range of diseases from ALS to Alzheimer's disease to stroke.

The majority of these trials are in spinal cord injury and use autologous MSCs for treating these disorders. Also, most of the trials are in phase 1/2 mainly assessing the safety of these cells to treat neuronal disorders.

19.3.1 MSCs in Stroke

The clinical trials database lists 3 trials using MSCs for stroke. The trial by Stemedica Technolgies in San Diego uses autologous MSCs for treating ischemic stroke and is currently ongoing. A trial by Stempeutics Research Malaysia (NCT01461720) will use cultured allogenic MSCs in stroke patients and will start recruiting patients soon. A trial in China (NCT01461720) is utilizing allogenic MSCs in the treatment for stroke. A larger proportion of trials in stroke use allogenic MSCs compared to MSCs in other disorders. This might be due to the immune privileged environment in the brain.

19.3.2 MSCs in Spinal Cord Injury

There is lot of preclinical and limited clinical data showing the beneficial effects of MSCs in treating spinal cord injury [8–11]. Therefore most clinical trials involving use of MSCs are in the area of spinal cord injury. Of the 6 trials listed for spinal cord injury, 4 trials are using bone marrow-derived MSCs for treating spinal cord injuries, the trial from RNL Bio Korea uses adipose tissue MSCs while a phase II trial from China uses allogenic umbilical cord-derived MSCs.

19.4 MSC Trials in Bone and Cartilage Disorders

Since MSCs can differentiate into bone and cartilage, these disorders were one of the earliest targeted for MSC therapy. The NIH clinical trials registry lists 22 trials using MSCs for treating bone and cartilage disorders. Of these, most of the trials are for treating osteoarthritis of knee joints or hip. Bone marrow MSCs are also being investigated to treat osteogenesis imperfecta. Two studies are also using autologous MSCs for treating nonunion fractures that are difficult to heal otherwise. A trial at St. Judes Hospital is using allogenic MSCs to treat osteodysplasia. The use of Cartistem, a umbilical cord-derived MSC product [12] by Medipost of South Korea is being evaluated for the treatment of microfractures. MSCs were used as vehicles for treatment [13] or compounds regulating MSC differentiation like oxytocin [14] have been proposed for treating osteoarthritis. Stempeutics in India and Cytopeutics in Malaysia are investigating the use of

MSC transplantation in treating osteoporosis. MSCs have also been used in treating cartilage disorders particularly in injured cartilage or degenerative joint disease [15, 16].

19.4.1 Use of Scaffolds for Tissue Engineering of MSCs

Many of these trials involved growing MSCs on scaffolds that mimic the mechanical properties of the tissue being targeted. For bone this usually involves an inert hard surface that supports the growth of MSCs [17–20]. For cartilage materials like collagen [21], chitosan [22] or extracellular matrix-derived scaffolds [19] have been used to seed MSCs. A good summary of different scaffold materials used in tissue engineering can be found in reviews by Boo, Warren and Gigante [20, 21, 23].

19.5 MSCs as Immunosuppresants in Graft-Versus-Host Disease

Since MSCs do not express MHC Class II molecules on their surface and act as immunosuppresants, these have been used in the treatment of graft-versus-host disease (GVHD). The clinical trials database lists 15 trials involving the use of MSCs in treating GVHD. The drug Prochymal is an MSC product developed by Osiris Therapeutics for treating GVHD [24]. Its role has been investigated in treating GVHD especially in patients where the condition is steroid refractory. Apart from the Osiris studies, trials investigating the use of MSCs for treating GVHD are being carried out in Spain, China, Korea, India, and Belgium.

Apart from GVHD, MSCs are also being investigated in the treatment of other autoimmune disorders like lupus and Crohn's disease. Nine trials have investigated the use of MSCs in Crohn's disease, an immune linked disorder of the gastrointestinal system. Most of these trials are phase II/III indicating the promising result from phase I studies. Companies like Cytomed and Beike are also investigating the use of MSCs in treating lupus in phase II trials.

19.6 MSCs in Cancer Therapy

MSCs have been used in the management and treatment of cancers in two ways. The first is use of MSCs for increasing engraftment in HSC transplants in hematological malignancies. In addition to reducing GVHD [25, 26], MSCs also aid in the engraftment

of donor cells [27]. Co-transplantation of MSCs as facilitating cells also preserves the desirable graft v/s tumor effect [28].

19.7 MSCs for Treating Liver Disease

MSCs are being investigated extensively in treating liver disorders. The NIH database lists 18 trials investigating the use of MSCs in treating liver disorders. Most of these are liver failure due to cirrhosis or in some cases fibrosis. MSCs are used to augment the regenerative process in the liver or to directly replenish hepatocytes. Since MSCs can differentiate into hepatocytes in a number of preclinical animal models [29–32], the current trials investigate the safety and efficacy of this treatment in humans. Readers are recommended to the detailed reviews by Christ and Gilgenkranttz that summarize the impact of MSCs in hepatic disorders [33, 34].

19.8 Critical Limb Ischemia and Buerger's Disease

MSCs have been shown to play an important role in wound healing and therefore have been used in treating lower limb ischemias, foot ulcers as well in Buerger's disease [35]. The NIH clinical trials database lists six studies investigating the use of MSCs in treating critical limb ischemia or Buerger's disease. This is especially pertinent to countries like India, where it is common for people with these disorders to walk barefoot. Thus two such trials are being carried out by Stempeutics in India. Trials in Germany, Spain and Malaysia are also investigating bone marrow and adipose tissue-derived MSCs for treating critical limb ischemia.

19.9 MSCs in Diabetes

MSCs are used in directly treating insulin-dependent diabetes or diabetes-related limb ischemia [36] or foot ulcers. Trials at University of Sao Paulo and Qingdao University are investigating the use of MSCs in treating diabetes mellitus. Since MSCs are used in treatment of wounds and ulcers, the utility of these cells is also being examined in the treatment of such ulcers and wounds in diabetic patients in a number of trials. MSCs have been the front-runners relative to other stem cell types and are highly represented in clinical trials. Whether they could be effective in treating autoimmune disorders such as type 1 diabetes is still a question and remains to be established whether these cells can repair, replace, or restore the function of beta islet cells secreting insulin.

19.10 MSCs in Cell Therapy: Emerging Issues

19.10.1 Protocols

Cell therapy protocols require careful isolation of cells, expansion, differentiation, cryopreservation and preparation of transplant ready cells for delivery and meet the expected safety and efficacy, prior to their use in patients. Designing clinical trials and interpreting data from these trials is another challenge that cannot be underestimated. The first challenge is defining MSCs. Many trials use different ways to characterize and define MSCs making it difficult to compare data across trials. In 2006 the ISCT came up with a definition of MSCs and all trials using MSCs therapeutically are expected to define MSCs according to this definition. The other challenges are more generic to all cell-based therapies. It is difficult to prove safety and efficacy of these cells. As was evident with the use of MSCs in cardiac disorders, earlier promising results from small-scale studies may not hold up in larger randomized trials. Also some adverse effects of transplanted MSCs may be felt after many years (even decades) making long-term follow-up of these patients very important. Further it could take many years for transplanted MSCs to clear from the recipient. This is very different from traditional drugs where clearance is rapid and the amount of drug cleared from the recipient's system can be precisely measured. The other major challenge for use of MSCs in the clinic (especially in the development of large batches of MSCs for allogenic use) is the difficulty of establishing chemistry manufacturing controls for MSCs. These are standard controls for pharmaceuticals that characterize the drugs and ensure that the administered drug will behave in vivo as expected. Making such accurate predictions and such precise characterizations for MSCs is often very difficult or impossible. For example, even if we are able to characterize MSCs accurately, how do we predict exactly how transplanted MSCs will behave in vivo? This requires newer chemistry manufacturing controls defined for cell-based therapies including MSCs which would be different from such controls currently used for drugs.

19.10.2 Controlling Differentiation of Transplanted MSCs in Various Tissues

In a clinical study it is important to assess the safety and efficacy of the drug being administered. For MSCs, this means assessing the toxicity of transplanted MSCs and predicting the fate of these cells. It is important to achieve targeted differentiation of MSCs into the desired lineage only. It is extremely difficult to achieve such targeted differentiation of MSCs in vivo. Differentiation into undesired lineages may contribute to the toxicity of these cells. Differentiation of transplanted MSCs into fibroblasts in the heart is an example of such differentiation into an undesired lineage. This also applies to MSCs seeded on devices that may be transplanted. For

example, MSCs seeded onto 3D scaffolds for transplantation into cartilage may differentiate into bone cells which could cause toxicity of the implants. Understanding the precise cellular mechanisms governing MSC differentiation thus becomes very important [37].

19.10.3 Expanding MSCs in Large-Scale Cultures Under cGMP Conditions

Although MSCs can expand to a large extent without differentiating, their expansion capacity is often limited. MSCs have been expanded in bioreactors [38–40]. For therapeutic applications it is essential to expand MSCs in a closed system [41] under xeno-free conditions [42–44]. The development of serum-free media for expanding MSCs [45] led to the development of xeno-free protocols for MSC expansion. This is essential for the widespread adoption of MSC-based therapeutics. However these media and MSC isolation procedures are expensive and this remains the single most important hurdle in the mass adoption of MSC-based therapeutics.

19.11 Summary and Conclusion

In summary, MSCs are being used in a variety of disorders to treat multiple diseases. Many of these trials are ongoing and some are recently completed. Thus the findings are still being analyzed or are not publicly available. The long-term fallout of using MSCs as therapeutics still remains uncertain, while the early results are quite promising. A recent study by von Bahr [46] demonstrates that MSCs are cleared rapidly in recipients of MSC infusions undergoing hematopoietic stem cell transplants. Thus this study concludes that the long-term risk of MSC transplantation is limited since the cells are cleared rapidly in the recipients. Such studies of safety coupled with preliminary studies showing efficacy form the basis for future large randomized trials. Such studies will conclusively ascertain the benefits of MSCs in specific disorders. It is also possible that in many trials variables like cell dose, route and frequency of administration, stage of disease, etc. will have to be optimized before we can see the beneficial effects of MSCs.

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