Chapter 8
Stem Cell Therapy: From the Heart to the Periphery

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Abstract Peripheral artery disease remains a clinical challenge—together with coronary artery disease, it accounts for increased morbidity and mortality in the concerned patients. Therapeutic concepts are often limited because of underlying co-morbidities and generalised atherosclerosis. The search for new forms of intervention follows several directions with stem cell therapy or therapeutic angiogenesis being one of the most promising approaches. The following chapter should provide an overview on the significance of the disease and the limitations of currently applied procedures. The biological concept which is the driving force of improvement in this special clinical situation is presented, and a brief overview on the history of stem cell therapy for vascular regeneration is given. So far, regarding peripheral artery disease, this story is a story of success, and future clinical approaches will take into account new sources of stem cells beside bone marrow to successfully treat patients with the disease, even in palliative situations.

Keywords Peripheral artery disease • Therapeutic angiogenesis • Revascularisation • Ischaemia • Perfusion

8.1 Introduction

Stem cells of variable sources have demonstrated significant potential for vascular regeneration in peripheral arterial disease. Preclinical studies proved beneficial effects in animal models of critical limb ischaemia in terms of revascularisation, and clinical trials showed clear advantage in stem cell-treated patients with critical limb ischaemia with regard to symptoms and wound healing. Because many of these

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patients are not eligible for revascularisation or endovascular procedures, there is an urgent need for novel therapies to improve the clinical situation and the quality of life. These no-option patients are treated in clinical trials with stem cell preparation of various sources like bone marrow, peripheral blood, or adipose tissue.

8.2 The Burden of the Disease

Coronary (CAD) and peripheral (PAD) arterial diseases are major causes of morbidity and mortality around the world, and millions of patients with CAD and PAD are treated by different medications, bypass surgery, or angioplasty. Risk factors for atherosclerotic PAD are mainly, but not exclusively, smoking and diabetes and are, therefore, comparable with those for atherosclerosis in the cerebrovascular and coronary circulation. Atherosclerotic occlusive disease of the lower extremities is the most frequent form of atherosclerosis, and PAD is a strong marker for atherothrombotic disease in other vascular beds [1]. PAD is present in approximately one-half of all patients with foot ulcers accounting for chronic wounds due to insufficient perfusion [2]. Along with polyneuropathy, PAD causes foot ulceration which is the major cause of non-traumatic lower-limb amputations. As a rather mild form of PAD, intermittent claudication (IC) is characterised by pain upon walking, limiting the pain-free walking distance. Chronic ischaemic rest pain, ulcers, or gangrene attributable to objectively proven arterial occlusive disease characterise critical limb ischaemia (CLI) which is the most advanced and severe form of PAD. Only 20 % of patients with critical limb ischaemia describe previous symptoms of intermittent claudication [3]. PAD is estimated to develop in 500–1,000 individuals per million persons in the general population; the prevalence of all stages of PAD in the general population is estimated to be 4.2–35 % and varies by country [4, 5]. Progression of PAD towards CLI is expected to occur for 4.3–9.6 % of the PAD patients, eventually resulting in amputation of the affected limb [3, 5]. For Germany, data are available from the observational German Epidemiological Trial on Ankle Brachial Index (getABI study) from 2004, in which the ABI of consecutive, unselected patients aged 65 years or older with bilateral Doppler ultrasound measurements was determined. A total of 6,880 patients were included (42.0 % male, mean age 72.5 years). The prevalence of PAD for men/women as indicated by an ankle-brachial index (ABI) <0.9 was 19.8 %or 16.8 %, respectively [6]. In the US population, the prevalence of PAD was 4.5 % in the general population but increased to 9.5 % in persons with diabetes [7]. PAD develops at a younger age among patients with diabetes as compared to the general population [8]. Diabetes mellitus causes almost 50 % of all non-traumatic amputations of the lower extremities worldwide, and more than 80,000 procedures are performed annually [7]. The lifetime risk for amputation in diabetic patients is 10–15 %, which is 10–30 times higher in comparison to the general population [9–12]. Leg amputation due to atherosclerotic PAD corresponds to a mortality rate of around 30 % and a 5-year prognosis with survival rates of less

than 5 years [12–16], and even asymptomatic PAD by itself is a significant predictor of cardiovascular morbidity and death [17]. The presence of cardiovascular risk factors and co-morbidity importantly contributes to the reduced survival. CLI has important functional implications, and its impact on the quality of life, assessed as quality of life indexes, has been reported to be similar to those of terminal cancer patients [18, 19]. In addition, CLI is associated with higher numbers of surgical interventions and hospitalisation [20, 21].

8.3 Treatment Concepts and Why They Do Fail

Despite therapeutic and technical advances in endovascular surgery, CLI continues to be associated with a high risk of (cardio)vascular events, including major limb loss, myocardial infarction, acute coronary syndrome, stroke, and death, especially in patients with diabetes mellitus [22–24]. Due to the widespread nature or the distal location of the occlusions and due to the presence of co-morbidities, PAD patients are at highest risk for peri-procedural complications and death. Because of the high operative risk or unfavourable vascular involvement, up to 40 % of patients do not qualify for such surgical interventions or endovascular procedures [25–27]. Thirty percent of patients undergoing amputation previously underwent one or more revascularisation attempt [28]. This accounts for about 100,000 major leg amputations in the European Union, and 120,000 in the United States [13, 29]. There is an unmet need for new strategies to offer these patients an additional and viable therapeutic option. The prognosis of death is around 20 % within 6 months of CLI diagnosis and rises to more than 50 % at 5 years after diagnosis [13, 30]. These extraordinary high mortality rates exceed those seen in any other pattern of occlusive disease like symptomatic coronary artery disease [1, 31] and reflect the severity of systemic effects associated with a diagnosis of CLI.

Treatment decisions in CLI are individualised and should take into account life expectancy, functional status, anatomy of the arterial occlusive disease, as well as surgical risk and are often multidisciplinary. Open surgical bypass was regarded as the most effective treatment strategy for limb revascularisation in these patients for a long time. Endovascular procedures treatment options were improved and are part of clinical routine in the angiologic treatment of PAD. Multimorbidity of the patients with extensive co-morbidities (atherosclerosis or heart disease), the anatomic location of the lesion, or the extent of the disease limit surgical interventions, and in subgroups primary amputation remains the only treatment option. Perioperative mortality in 5–20 % of the patients accounts for the bad prognosis of amputation as well as the risk for a second amputation in 30 % of cases, with only 25–50 % of subjects achieving full mobility [26]. The median cost of successful limb salvage is half of the costs for the management of a patient after amputation [26, 32]. These patients with no option of either surgical or endovascular revascularisation might benefit from stem cell therapy and/or tissue engineering strategies that aimed at

accelerating the natural processes of vascularisation, angiogenesis, and tissue repair [33]. Several clinical studies reveal that the injection of bone marrow-derived mononuclear cells (BMC) results in improvement in symptoms and healing of ulcers in patients with CLI up to stage IV of Fontaine's classification [34].

8.4 Basics of Vessel Formation and Biology

The key steps in vessel formation comprising endothelial cell activation, migration, proliferation, and reorganisation are highly regulated in a complex balance of pro- and anti-angiogenic mechanisms. The de novo synthesis of blood vessels from endothelial progenitors which differentiate into endothelial cells and fuse into luminal structures is called vasculogenesis. During embryonic vascular development, pluripotent stem cells differentiate to endothelial cells, which upon development form a primitive vascular network by assembly, called the primary capillary plexus. Vascularisation of several organs, like the endocardium of the heart and the dorsal aorta, occurs by vasculogenesis. In adult neovascularisation, migration and differentiation of bone marrow-derived endothelial progenitor cells (EPCs) are involved [33, 35]. Hypoxia and the key transcriptional system hypoxia-inducible factor (HIF) are major inductors for both angiogenesis and vasculogenesis by enhancing the synthesis of pro-angiogenic factors like vascular endothelial growth factor (VEGF), angiopoietin, and inducible nitric oxide synthase (iNOS) [36–38].

Arteriogenesis refers to an increase in the diameter and calibre of pre-existing arteriolar collateral connections. Perivascular cells are recruited within this process, and expansion and remodelling of the extracellular matrix occurs. Arteriogenesis results in the increase of collateral vessel size and wall thickness with shear stress rather than hypoxia being the main stimulus of arteriogenesis [39]. Shear stress leads to an upregulation of cell adhesion molecules for circulating monocytes, which subsequently accumulate around the proliferating arteries and provide the required cytokines and growth factors [38, 40].

Angiogenesis is defined as the sprouting of new capillaries from an existing vascular structure, a process that is triggered by endothelial cell migration and proliferation. Remodelling of the extracellular matrix (ECM), tubule formation, and expansion of the surrounding vascular tissue as well as remodelling of newly formed vessels into 3-dimensional networks with regression of unnecessary microvessels are key elements of angiogenesis. Angiogenesis occurs as a sprouting of small endothelial tubes from pre-existing capillary beds in response to local hypoxia. It is mediated by hypoxia-induced release of cytokines like VEGF and related growth factors [41]. The resulting capillaries are rather small, with a diameter of about 10–20 μm, and cannot sufficiently compensate/substitute for a large occluded transport artery. Organs like the brain, the kidneys, and the developing limbs are vascularised by angiogenesis. Angiogenesis is likely to be the very first mechanism for most new blood vessel growth in the adult, regardless whether it is a result of physiologic or pathologic stimuli like cancer growth [42, 43].

8.5 Role of Stem Cells in Therapeutic Angiogenesis: Manufacturing and Biological Challenges

Stem cells have demonstrated significant potential for regeneration in peripheral arterial disease in both animal and human studies. While results of clinical trials have been variable with respect to myocardial infarction and dilated cardiomyopathy, they have clearly proven benefit for patients with critical limb ischaemia and peripheral arterial disease [34]. Preclinical trials have demonstrated the angiogenic and vasculogenic potential of autologous bone marrow-derived stem cells (BMCs) in the treatment of PAD [44–47]. BMC are preferred in the cellular therapy of vascular diseases since bone marrow can be easily accessed, is renewable, and is an autologous source for regenerative cells. The use of purified and selectively expanded cell populations may allow a more target organ-specific stem cell therapy in the future. For therapeutic purposes, 50–250 ml adult bone marrow blood is aspirated from the iliac crest under local anaesthesia [34]. Mononuclear cells are separated from the whole bone marrow aspirate by density gradient centrifugation [48]. To overcome open preparation procedures and the application of several washing steps, newer protocols apply closed-tube procedures which reduce contamination risk. In summary, good manufacturing practice processes to produce a quality-controlled and contamination-free cell product [49, 50]. During cell preparation, viability needs to be determined several times and finally must reach approximately 95 % to guarantee functionality. Cell product characterisation by fluorescence-activated cell sorting or a cell counter is needed for product release. This manufacturing process can be performed within one working day in an ambulatory setting.

BMCs comprise several cell populations having the capacity to proliferate, migrate, and also differentiate into various mature cell types, best analysed in the application after myocardial infarction. Among these cells are haematopoietic stem cells [51–54], mesenchymal stem cells [55, 56], endothelial progenitor cells [57, 58], and side population cells [59, 60]. The angiogenic properties of BMCs have been attributed to the differentiation of these pluripotent stem cells into endothelial cells, thereby generating new blood vessels [61]. In addition, BMC releases cytokines and growth factors that promote angiogenesis. BMC releases vascular endothelial growth factor (VEGF), and the chorioallantoic membrane is an ischaemic environment, stimulating vasculogenesis [62, 63]. The most important cell populations involved in angiogenesis are CD133+ cells [64], CD117+ cells [65], and CD34+ cells [66], in addition to the mesenchymal stem cells [67].

Endothelial progenitor population comprises a heterogenous population of cells such as CD34-/CD133+/VEGFR2+ and CD34+/CD133+/VEGFR2+, in addition to the mature endothelial cells. The CD34-/CD133+/VEGFR2+ fraction is the precursor of the CD34+/CD133+ population and shows more potent vasoregenerative capacities [68, 69]. Endothelial progenitors are reported to be mobilised by several agents such as chemotherapeutic agents [70], metronomic chemotherapy [71], and erythropoietin [72]. These agents can be used therapeutically either to enhance angiogenesis in ischaemic cases or to reduce angiogenesis in cases of malignancies.

8.6 Working Concepts

New vessel formation to improve tissue perfusion through the three mechanisms vasculogenesis and/or angiogenesis in the ischaemic tissue as well as collateral vessel formation via arteriogenesis is a main topic therapeutic neovascularisation relies on. Positive effects on classical perfusion markers like TcPO2 could be clearly demonstrated. Physiological effects like collateralisation or angiogenesis have scarcely been described and could not always be attributed to clinical success [73].

Whether these effects can be attributed to the incorporation of stem cells into the wall of the new vessel, or homing stimuli released by platelets, or to the cytokines released by chemo-attracted BMCs inducing proliferation of resident endothelial cells remains an open issue. Imaging techniques to follow up with the injected cells are improving and deliver first results in animal models but also prove a diminished survival of the cells [74, 75]. An interesting finding is that hypoxia induces progenitor cell mobilisation through HIF-1α induction of SDF-1 and controls subsequent differentiation into endothelial cells through HIF-1α-regulated VEGF expression [36, 37, 76]. Mesenchymal stem cells mobilise to sites of ischaemia and adopt a partial endothelial phenotype when exposed to similar vasculogenic stimuli such as hypoxia. Mesenchymal stem cell recruitment and subsequent endothelial differentiation within ischaemic tissue may indeed be driven through the HIF-1α/SDF-1/VEGF pathway. Kinnaird et al. were able to show that cultured human BM-derived stromal cells promote arteriogenesis through paracrine mechanisms [77]. This concept is supported by Heil et al., who suggest that in the adult organism, bone marrow cells (BMCs) do not promote vascular growth by incorporating into vessel walls but rather act as “cytokine factories or depots”, promoting vascular growth by paracrine effects [78]. Findings by Jin et al. also support this concept by which ischaemia induces plasma elevation of stem and progenitor cell-active cytokines, including sKitL (Soluble Kit-ligand) and thrombopoietin, and, to a lesser extent, progenitor-active cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and erythropoietin [79].

Based on the experience from the application of stem cells in heart diseases, it is known and estimated that the regenerative potential of bone marrow-derived stem cells may be explained by at least any of 4 mechanisms [80]: (1) transdifferentiation of BMCs to cardiac myocytes [81, 82]; (2) cytokine-induced myocyte growth [83, 84] induced by cytokines (like vascular endothelial growth factor, insulin-like growth factor, platelet-derived growth factor) and increase of residual viable myocytes (especially in the zone of the infarcted area); (3) stimulation of intrinsic myocardial stem cells (endogenous stem cells) [83]; and (4) induction of cell fusion between transplanted BMCs and resident myocytes [85, 86], which was taken as an explanation for transdifferentiation.

Precise mechanisms cannot be given; it seems to be a cocktail phenomenon that boosts the effect—in the heart as well as in the periphery.

8.7 Cellular-Based Therapeutic Concepts in PAD/CLI

Therapeutic angiogenesis using stem cells, autologous progenitor cells, growth factors such as basic fibroblast growth factor, and transcription factors such as hypoxia-inducible factor-α that induce synthesis of angiogenic cytokines have been used in critical limb ischaemia patients who lack options for endovascular or surgical revascularisation [34]. Single growth factor therapy proved to be insufficient in the treatment of CLI [87], whereas cellular-based therapies are reported to be successful at various study sites [34]. The fact that bone marrow cells are composed of extensive complex cell fractions containing many kinds of undifferentiated stem cells and differentiated cells obviously guarantees for successful application. Implantation of autologous bone marrow cells is proven to be an effective and feasible technique of inducing therapeutic angiogenesis in both clinical and experimental studies. However, the angiogenic potency might differ among cell fractions of bone marrow cells, and which of these play a key role is yet unclear.

Injection of unfractionated bone marrow mononuclear cells has been reported to promote neovascularisation of ischaemic tissues effectively. This angiogenic effect may be related to their ability to induce vascular and muscle regeneration by direct de novo vascular and muscle differentiation or paracrine mechanisms through vascular endothelial growth factor secretion as described before. The working concept of BMCs in humans cannot be answered finally. Some but not all studies report neovascularisation and angiogenesis during treatment which is supposed to trigger wound healing in chronic wound situations like diabetic foot [48, 73].

Our recently published results of the prospective clinical trial to evaluate the safety and efficacy of non-expanded and expanded bone marrow-derived mononuclear in the case of diabetic critical limb ischaemia prove the safety and functionality of stem cell treatment in this population [73]. The study enrolled critical limb ischaemia patients who were no-option cases. A typical example is given in Fig. 8.1, showing a patient who was successfully treated with bone marrow-derived mononuclear cells [48, 73].

The route of administration of stem cells was by intramuscular or intra-arterial injection. The cell product was injected in the calf muscle or infused in the arteria femoralis. We used 40–50 ml bone marrow as starting material which was expanded over a time period of 12 days leading to an accumulation of mesenchymal stem cells and compared this cellular product to bone marrow cells. Currently, three routes of stem cell administration in critical limb ischaemia are applied: intramuscular, intra-arterial, or a combination of both. With the intra-arterial administration into the common femoral artery of the ischaemic leg, mononuclear cells are supposed to reach the region of maximum ischaemia by blood flow [44, 88]. While travelling in the circulation, nutrient and oxygen supply are preserved and provide a favourable environment for survival and engraftment, but the uptake from the circulation may be limited and cells may be damaged and loose potential due to shear stress. In this type of delivery, homing requires migration of cells out of the vessels into the

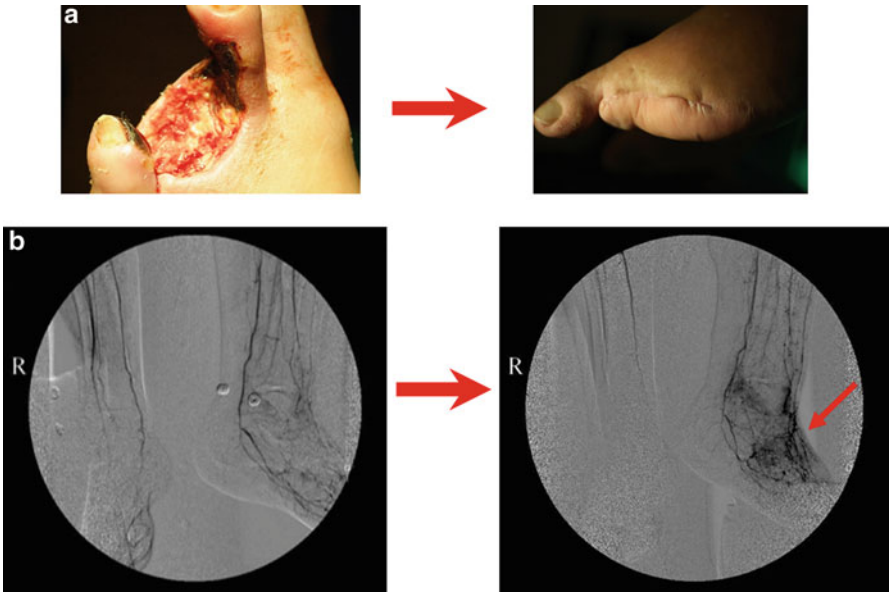

Fig. 8.1 Use of autologous bone marrow stem cell therapy leads to clinical improvement and 100 % wound healing in a patient with peripheral arterial occlusive disease. (**a**) *Left*: the situation at treatment beginning is shown, *right*: after 20 weeks. (**b**) Fine needle angiogram before autologous bone marrow stem cell therapy (*left*) and 20 weeks after the therapy (*right*), new collateralisation of capillary vessels of the forefoot is highlighted by the *arrow*

surrounding tissue, which makes ischaemic tissue targets less efficiently [89]. The intramuscular injection of stem cells with creation of a cell depot with paracrine activity in the ischaemic area overcomes this issue, but it is less clear what the fate of these muscle-deposited cells is [38]. The survival of the cells may be decreased, because of the injection site being nutrient and oxygen depleted [89, 90]. In our trial, outcome was not related to the application route, and there was no clear benefit of expanded cells, which were enriched in mesenchymal cells [73].

Most studies on cell therapy for critical limb ischaemia used the entire mononuclear cell fraction. Angiogenic effects of bone marrow mononuclear cells and peripheral blood mononuclear were shown to be equal in stimulation of neovascularisation [91]. The dose of infused cells plays a pivotal role in the effectiveness of therapy in terms of time to effect. Typically, the total cell number of mononuclear cells ranged from 0.1 to 101×109 cells [92–94]. The fraction of CD34+ cells in the isolated mononuclear cells population varies from 0.6 to 2.4 % in the therapeutic angiogenesis using cell therapy studies [95, 96]. Classical parameters to prove success is reduction in ischaemic pain, improved walking distance, improved ankle-brachial index and transcutaneous oxygen pressure, and signs of wound healing. Some studies also prove therapeutic effects by angiography [34, 73, 97, 98].

8.8 The Beginning: Preclinical and Clinical Applications

Preclinical studies showing that application of BMCs, including EPCs, into ischaemic limbs increases collateral vessel formation led to the evaluation of safety and feasibility of these cell-based therapies in patients with PAD. Clinical benefits can be noticed in form of a decrease in ischaemic symptoms and an increase in ankle-brachial index, transcutaneous oxygen pressure index, and exercise tolerance. The working hypothesis is that the observed clinical effects are associated with an improvement in angiogenesis, formation of new collaterals and/or augmentation of endothelium-dependent vasodilation. It is beyond the scope of this contribution to comment and review all trials done so far; there are excellent and actual reviews which the reader is referred to [34, 99, 100].

The Therapeutic Angiogenesis by Cell Transplantation (TACT) study by Tateishi-Yuyama et al. was the first larger study on the use of BMC in limb ischaemia [96] and delivered the concept for many other following studies. It was an open pilot study in which efficacy and safety of autologous implantation of BMC was established and a randomised controlled confirmatory part, comparing the efficacy of BMC vs. peripheral blood PBMC treatment. In the PBMC part, patients with bilateral leg ischaemia were randomly injected with BMC in one leg (active treatment) or with PBMC into the other as a control. At 4 weeks, ankle-brachial index (ABI) was significantly improved in legs injected with BMC compared with those injected with PBMC. Similar improvements were detected for transcutaneous oxygen pressure, rest pain, and pain-free walking time. Legs injected with PBMC cells showed much smaller increases of ABI and TcPO2. The improvements in the BMC-injected legs were sustained at 24 weeks [96]. The TACT study served as a basis for many other protocols of comparable studies which proved safety and feasibility of BMC treatment in CLI. The use of BMC instead of PBMC is more favourable due to the fact that bone marrow punction is more rapidly and less variable in terms of quality and number of cells. PBMC collection requires expensive G-CSF injections over 5 consecutive days and plasmapheresis for several hours, making this process costly and time-consuming. Lu et al. were able to prove in a double-blind placebo-controlled trial that bone marrow-derived mesenchymal stem cells are more potent than bone marrow mononuclear cells in terms of time to wound healing, painless walking time, ABI, TcPO2, and magnetic resonance angiography (MRA) analysis. No difference was detected regarding pain relief and amputation rate [101].

Since the TACT publication, there have been more than 30 reported therapeutic cell trials in patients with PAD. Recently, Fadini et al. did a review of the literature searching for effective autologous cell therapy studies for the treatment of PAD [34]. They found 108 reports, of those 42 were clinical trials and 37 fulfilled the criteria to be meta-analysed. In general cell therapy was effective in improving surrogate indexes of ischaemia, subjective symptoms, and hard end points (ulcer healing and amputation). In all trials with data on ankle-brachial index and transcutaneous oxygen pressure, ABI and TcPO2 improved significantly during therapy;

regarding only controlled trials, the effect on ABI was smaller, but still significant, whereas the effect on TcPO2 was not detectable with statistical significance. Increase in walking capacity and pain relief was demonstrated in all trials. Ulcer healing significantly improved in the active treatment group vs. the control group in controlled cell therapy trials. Amputation rate was documented in only two controlled trials of cell therapy indicating a significant benefit in terms of limb salvage as compared to control treatment. This meta-analysis demonstrates that cell therapy is able to significantly improve ABI, TcPO2, rest pain, pain-free walking distance, ulcer healing, and limb salvage but the physiological explanation remains obscure.

The National Institute of Health publishes clinical trials that have been approved by the Food and Drug Administration, European medicines agency, and other national regulatory bodies under an identification number and activation status in www.clinicaltrials.gov as shown as of September 2012. Currently, 34 studies are listed if "critical limb ischemia and stem cells" are entered as search terms and results are controlled for inclusion criteria. Of those, 9 studies are recruiting patients, 14 are completed, 5 are not recruiting, and the remaining 4 studies have an unknown status. These studies will deliver more knowledge on the effect of this therapy. Current studies prove that cellular therapy is well tolerated and offers rising hope for patients with peripheral arterial disease. Administration of autologous bone marrow mononuclear cells is easy to perform, inexpensive, and safe, with a definite ameliorating effect on limb ischaemia. However, specification of the target cell population, route of administration, and dose escalation needs to be evaluated in larger case-controlled studies.

8.9 Future Directions

Several sources of autologous stem cells have been tested in preclinical and clinical trials: adipose-derived stem cells (ASC), bone marrow-derived mesenchymal stem cells (BM-MSC), human umbilical vein endothelial cells (HUVEC) and embryonic stem cell-derived endothelial cells (ESC-EC), embryonic stem cell-derived mesenchymal stem cells (ESC-MSC), and induced pluripotent stem cell-derived endothelial cells (iPSC-ECs). Since iPSCs can be derived from a variety of tissues and have high replicative capacity, they are potentially an unlimited source of autologous therapeutic cells. Preclinical studies using iPSC-derived cells have shown promise for treatment of sickle-cell anaemia, Parkinson's disease, ß-thalassemia, and peripheral arterial disease (PAD) [102–105]. Genetic and epigenetic abnormalities are the main issues raised against iPSC-ECs but may be obviated with careful generation, culture, and selection of iPSCs [106]. In addition to safety concerns, there are manufacturing hurdles to overcome for therapeutic application. Current reprogramming methods are inefficient, although reprogramming methods continue to improve. Whether these cell types will replace currently available clinical routines or will remain further alternative remains to be seen.

Stem cells are a promising reagent for vascular as well as tissue repair, but many obstacles need to be overcome before they can be widely used in clinical routine. Imaging techniques have pointed to the problem of limited cell viability of transplanted cells in ischaemic tissues that remains a major concern. Although the studies suggest that the cells can exert a therapeutic effect even without prolonged survival, strategies to enhance viability by using survival factors (i.e. HIF1α, Akt, bcl2) are under development. The addition of soluble factors and three-dimensional extracellular matrices may further promote cell survival and/or angiogenesis and add a new quality to this up to now successful story.

8.10 Conclusion

Benefits were reported from clinical trials using different sources of stem cells in patients with PAD including improvement of ABI and TcPO2, reduction of pain, and lower major amputation rates. Nonetheless, large randomised, placebo-controlled, double-blind studies are necessary and are currently ongoing to provide stronger safety and efficacy data on cell therapy. Current literature is supportive of intramuscular (bone marrow) cell administration as a relatively safe, feasible, and possibly effective therapy for patients with PAD who have no option for conventional revascularisation.

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