The Neuroscientist
2015, Vol. 21(6) 637–652

DOI: 10.1177/1073858414547538
nro.sagepub.com
SAGE

Bone Marrow–Derived Stem Cells and Strategies for Treatment of Nervous System Disorders: Many Protocols, and Many Results

David Díaz[1,2], Rodrigo Muñoz-Castañeda[1,2], José Ramón Alonso[1,2,3], and Eduardo Weruaga[1,2]

Abstract

Bone marrow stem cells are the best known stem cell type and have been employed for more than 50 years, especially in pathologies of the hematopoietic and immune systems. However, their therapeutic potential is much broader, and they can also be employed to palliate neural diseases. Apart from their plastic properties, these cells lack the legal or ethical constraints of other stem cell populations, that is, embryonic stem cells. Current research addressing the integration of bone marrow–derived cells into the neural circuits of the central nervous system, their features, and applications is a *hotspot* in neurobiology. Nevertheless, as in other leading research lines the efficacy and possibilities of their application depend on technical procedures, which are still far from being standardized. Accordingly, for efficient research this large range of variants should be taken into account as they could lead to unexpected results. Rather than focusing on clinical aspects, this review offers a compendium of the methodologies aimed at providing a guide for researchers who are working in the field of bone marrow transplantation in the central nervous system. It seeks to be useful for both introductory and trouble-shooting purposes, and in particular for dealing with the large array of bone marrow transplantation protocols available.

Keywords

bone marrow, cell therapy, methodologies, neurodegeneration, transplants

Introduction

Bone marrow stem cells (SC; Box 1) form the best-known population of the adult SC group (Table 1). They were identified more than 50 years ago as the cells responsible for the formation of blood cell populations (Becker and others 1963). They can also be classified in three subpopulations (Box 2): hematopoietic SC (Becker and others 1963), stromal cells (Friedenstein and others 1968), and side-population cells (Goodell and others 1996). Traditionally, stromal cells were considered to be the most plastic subpopulation, being capable of developing into the cells of several organs and tissues (Jiang and others 2002). However, after the late 1990s several laboratories discovered some surprising properties of hematopoietic SC, one of which was that they gave rise to hepatocytes (Petersen and others 1999) or myocytes (Ferrari and others 1998; Gussoni and others 1999). Finally, it has been demonstrated that hematopoietic SC (in fact, one single cell) could differentiate *in vivo* into elements of a broad variety of tissues (Krause and others 2001). In the late 1990s, it was also reported that bone marrow–derived cells (BMDC) could differentiate into cells of the central nervous system (CNS) *in vivo* (Eglitis and Mezey 1997). Three years later, two parallel works also demonstrated that BMDC could not only become glial cells but also neurons (Brazelton and others 2000; Mezey and others 2000). In the first reports, transdifferentiation was proposed as the underlying mechanism of this cell plasticity (Box 3; Brazelton and others 2000; Eglitis and Mezey 1997; Mezey and others 2000; Priller and others 2001). Later, however, different experiments revealed cell fusion as another mechanism of plasticity (Álvarez-Dolado and others 2003). Although these fusion events in neurons have only been detected in Purkinje cells, they deserve to be

[1]Laboratory of Neuronal Plasticity and Neurorepair, Institute for Neuroscience of Castilla y León (INCyL), Universidad de Salamanca, Salamanca, Spain
[2]Institute of Biomedical Research of Salamanca, IBSAL, Spain
[3]Instituto de Alta Investigación, Universidad de Tarapacá, Arica, Chile

Corresponding Author:
Eduardo Weruaga, Universidad de Salamanca, INCyL, C/Pintor Fernando Gallego 1, E-37007 Salamanca, Spain.
Email: ewp@usal.es

Table 1. Classification of Stem Cells (SC).

According to their potential	
Totipotent	The least differentiated SC, able to convert into all the cell types of both an organism itself and its extra-embryonic tissues. Totipotent SC comprise both the zygote and the embryo up to the first 2 to 3 divisions.
Pluripotent	These SC can give rise to any cell of an organism, but not those from extra-embryonic tissues. They constitute the inner cell mass of the blastocyst.
Multipotent	These can only generate new cells derived from their own germ layer. Also classified as progenitors or progenitor cells.
Unipotent	These can only form a single differentiated cell type.
According to their origin	
Embryonic	Derived from the embryo or the extra-embryonic tissues
From umbilical cord	Derived from the different tissues composing the umbilical cord
IPS cells (induced pluripotent stem cells)	Differentiated cells that have been genetically reprogrammed and dedifferentiated
Adult	Stem cells of adult organisms, commonly only multipotent or unipotent

taken into account because of their genetic implications (Álvarez-Dolado 2007; Yamanaka and Blau 2010). Therefore, both mechanisms—fusion and transdifferentiation—are not restrictive and coexist within the same animal (Recio and others 2011).

Bone marrow SC have been employed in clinical practice since 1956, when E. Donnall Thomas performed the first bone marrow transplantation in a patient with leukemia (Thomas 1983). Since then, the methodologies for therapeutic treatments employing bone marrow cells have been refined. In addition, and taking into account their unexpected plasticity, bone marrow SC (either as an nonfractionated cellular pool or as specific subpopulations) are currently being applied as a therapeutic tool in different models of disease and even in clinical trials: heart and coronary diseases (Porat and others 2014; Sadat and others 2014), infarctions (de Jong and others 2014; Henning 2013; Losordo and Vaughan 2014), stroke (Calió and others 2014; Jeong and others 2014), graft-versus-host disease (Herrmann and Sturm 2014; Li and others 2014), bone diseases (Ito 2014), cancers (Kikuchi and others 2014), or neural diseases (Kanno 2013), and not only those related to the hematopoietic system.

Box 1.

Stem cells

Stem cells (SC) are undifferentiated cells that possess a remarkable proliferative capacity to maintain tissue homeostasis and allow regeneration after injury. Two general properties are common to SC: self-renewal and an almost unlimited potential to differentiate. Regarding self-renewal, by means of symmetrical divisions, SC are capable of generating more SC identical to the original ones in theoretically limitless numbers. The differentiation potential refers to the ability of SC to change into other cell types of the organism they belong to.

Box 2.

Niches

The existence of different adult stem cell populations has been demonstrated in specific areas known as niches. These niches are located in the majority of adult organs and tissues. The cellular niche can be defined as the physiological microenvironment that surrounds and maintains a given SC population. However, the SC niche is much more than a merely special localization: niches are complex structures composed not only of SC themselves but also by neighboring cells, the extracellular matrix secreted by them, and the signals produced by both cell types. Niches are critical for controlling the proliferation and differentiation of SC, depending on the necessities of homeostasis or repair of the different tissues they belong to.

Box 3.

New properties of stem cells

Not all SC are capable of differentiating in all the different cell types of a given organism; the lower the differentiation state of a given SC, the higher the differentiation potential. However, several experiments have demonstrated that adult SC have unexpected properties that confer them a much greater potential than had previously been thought:

Transdifferentiation: the property of changing their natural fate for another one (i.e., a hematopoietic SC that gives rise to a neuron).

Dedifferentiation: to return backwards along the normal differentiation process, becoming more undifferentiated.

Cell fusion: to fuse with a differentiated cell (for a hitherto unknown purpose).

Regarding the use of bone marrow SC in research and possible therapies in the CNS, current methodologies are usually performed in mice (mainly) and rats, but lack standardization. Thus, we expect that this review will help researchers choose the most suitable variants of these techniques to achieve the best efficiency in their experiments. Depending on the focus of the experiment, different variables for bone marrow transplantation should be taken into account to avoid potential complications or false results. For the purpose of this review, the bone marrow transplantation protocol will be addressed in four separate stages: preparation of the recipient animal, preparation of the donor marrow, transplantation, and tracking and visualization of transplanted-derived cells. Finally, we have included a brief summary comprising the *pros* and *cons* of the clinical applications of BMDC, as well as some of the ongoing clinical trials employing these cells in neural diseases.

Preparation of the Recipient

Here it is necessary to discern between three classic types of bone marrow SC transplantation: direct engraftments into the encephalon (as well as in the cerebrospinal fluid), partial direct engraftments through the blood vessels (i.e., carotidal transplants), and a transplantation aimed at substitution of the recipient's bone marrow. The first method (direct engraftments) is a much more invasive methodology as it requires an opening to be made in the skull and subsequent injections into the brain, which will necessarily damage the neural parenchyma. Regarding the second methodology, whether intravenous or intraarterial, this is aimed at the direct delivery of the transplanted bone marrow into the brain through the vascular system. However, this methodology, although less invasive than the direct route of administration into the brain parenchyma, has the disadvantages of the retention of transplanted cells in different organs, or the formation of microemboli (Jiang and others 2011). Finally, bone marrow SC injections can be used—whether in blood vessels or intraperitoneal—aimed at the replacement of the bone marrow of the recipient, as it has been demonstrated in mice that BMDC can arrive physiologically at the encephalon and become neural cells (Brazelton and others 2000; Eglitis and Mezey 1997; Mezey and others 2000). However, this technique usually requires ablation of the recipients' own bone marrow to avoid rejection problems and to achieve an empty niche for the new labeled cells (Box 4; Fuchs and others 2004; Li and Xie 2005).

Box 4.

Homing

The capacity of an empty niche to attract—and also anchor and retain—its own SC. Homing is mediated through different chemokines, especially those related to inflammatory responses and host defense. Accordingly, some processes that lead to general inflammation, such as ionizing radiation, can accelerate the homing process. The cytokine-mediated signals and cell-adhesion and extracellular matrix molecules also play an important role in this capacity. Therefore, different chemicals aim at breaking these interactions to mobilize stem cells from their niches.

Additionally, a fourth transplantation methodology can be considered. This has been confirmed recently in mice and involves intranasal administration (Danielyan and others 2009). This almost noninvasive technique seems to ensure the direct arrival of engrafted cells into the encephalon, avoiding both their retention in other organs (as may occur with systemic injections) and ablation of the recipient's bone marrow (Danielyan and others 2009). Nevertheless, the integration of these cells into the encephalon as neural elements remains to be confirmed (see below).

In sum, bone marrow ablation—as preparation of the recipient—is only necessary if new marrow can replace it. In this sense, there are different possibilities of bone marrow ablation (Fig. 1A-C), including the possibility of not performing ablation at all.

Physical Ablation of Bone Marrow

Physical ablation involves the use of ionizing irradiation to destroy the bone marrow of a recipient (Fig. 1A). It is the most common ablative methodology, but it has different side effects (Díaz and others 2011; Eglitis and Mezey 1997; Recio and others 2011; Weimann and others 2003b; Wiersema and others 2007; and other laboratories). Thus, proliferating cells are susceptible to such radiation and indeed this is the basis of radiation therapy to treat tumoral or neoplastic disorders (Strother and others 2002). Accordingly, not only bone marrow SC but also other proliferating cells are killed by irradiation, including those whose niche lies inside the encephalon (Altman, 1969; Doetsch and others 1997; Romanko and others 2004). In this sense, physical ablation can cause secondary effects in the CNS. These effects differ, depending on both the brain proliferating region analyzed and the dose employed. Thus, radiation impairs neurogenesis both in the dentate gyrus of the hippocampal formation (Manda and others 2009; Monje and others 2002) and also in the subventricular zone (Tada and others 1999) of rodents, the former region being more

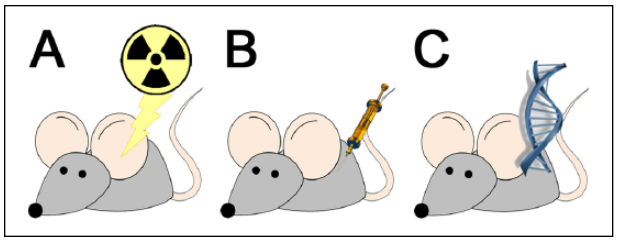

Figure 1. Different methodologies for bone marrow ablation. Bone marrow of recipients can be ablated by physical methods such as radiation (A); by injections of chemicals such as busulfan, treosulfan, or fludarabine (B); or by means of genetic modifications (C).

vulnerable than the latter (Hellstrom and others 2009; Mizumatsu and others 2003). In addition, radiation has a dose-dependent effect (Tada and others 1999), which varies from severe damage without recovery at higher doses (Díaz and others 2011; Shinohara and others 1997) to complete restoration (Hellstrom and others 2009), and it may even increase neurogenesis at lower doses (Balentova and others 2006; Balentova and others 2007). In any case, the minimal (lethal) dose of radiation necessary to ablate bone marrow—7.5 Gy—impairs adult neurogenesis in mice (Díaz and others 2011). In experiments employing irradiation to ablate the recipient's bone marrow and detect the integration of BMDC into the brain, the dose usually used is similar to this minimal dose (Álvarez-Dolado and others 2003) or even higher (Table 2). Accordingly, in all radiation applications higher than 7.5 Gy (even fractionated into several sessions of lower doses), neurogenesis is impaired. Radiation also affects encephalic regions other than neurogenic zones. For example, it can damage Purkinje neurons in newborn mice, but not in adults, even at low doses (Espejel and others 2009).

There is some controversy about the effects of radiation on the incorporation of BMDC, as some reports have shown that radiation is not necessary for the integration of BMDC into the intact encephalon of mice (Espejel and others 2009; Johansson and others 2008), and a similar number of bone marrow-derived neurons can be found after both chemical and physical ablation (Magrassi and others 2007). However, some of the side effects of radiation may be beneficial for the cells arriving at the brain (even for cell therapies); for example, it has been reported that radiation accelerates blood reconstitution in bone marrow–transplanted animals (Nygren and others 2008). In addition, in rats and mice injury in the encephalic parenchyma fosters the arrival and integration of BMDC, generating both neuronal and glial cells (Fig. 2; Díaz and others 2012b; Johansson and others 2008; Magrassi and others 2007; Recio and others 2011). Thus, radiation-derived damage also enhances the arrival of BMDC in the cerebellum, increasing the cell fusion process

Table 2. Doses of Radiation Employed for Bone Marrow Ablation.

4.5–5.5 Gy (multiple sessions)	Eglitis and Mezey 1997; Johansson and others 2008; Weimann and others 2003b; Priller and others 2001
7.5 Gy	Díaz and others 2011; Díaz and others 2012a; Díaz and others 2012b; Recio and others 2011: Álvarez-Dolado and others 2003
8 Gy	Corti and others 2002, Corti and others 2004; Rodríguez and others 2007
9.5 Gy	Massengale and others 2005; Chen and others 2011; Wiersema and others 2007
10–11 Gy	Moore and others 2005; Vallieres and Sawchenko 2003; Krause and others 2001

(Espejel and others 2009). Finally, radiation also facilitates BMDC incorporation (Wiersema and others 2007), presumably due to an opening of the blood-brain barrier (Yuan and others 2006).

In conclusion, radiation is an effective and widely employed methodology for bone marrow ablation. Although it is not necessary for the incorporation of BMDC into the brain parenchyma, it does seem to facilitate such integration. However, some caution should be exercised because of the damage of radiation to proliferating cells or to other brain regions that might modify certain parameters of an experiment.

Chemical Ablation of Bone Marrow

The second possibility for bone marrow ablation is the use of chemicals (Fig. 1B), which are also used in human chemotherapy against neoplasms (Locatelli and others 1994). Busulfan has been employed in clinical practice since the 1950s to perform such chemotherapy (Down and others 1994), although treosulfan (Scheulen and others 2000) and fludarabine (Casper and others 2004) are more recently introduced agents and have similar or even better pharmacokinetic and tumor-suppressive properties than those of busulfan (Ploemacher and others 2004; Sjoo and others 2006). All these chemicals have a highly selective toxicity and hence lack major secondary effects apart from the ablation of bone marrow, contrary to other alkylating drugs such as cyclosporamide, bischloroethylnitrosourea, or isopropylmethanesulfonate, which are also less effective (Down and others 1994).

The use of these drugs allows the ablation of bone marrow (and subsequent transplantation) in adult mice (Down and others 1994; Magrassi and others 2007; Sjoo and others 2006), but busulfan can be used to ablate bone marrow before birth (Espejel and others 2009; Piquer-Gil and others 2009). This latter possibility is very important

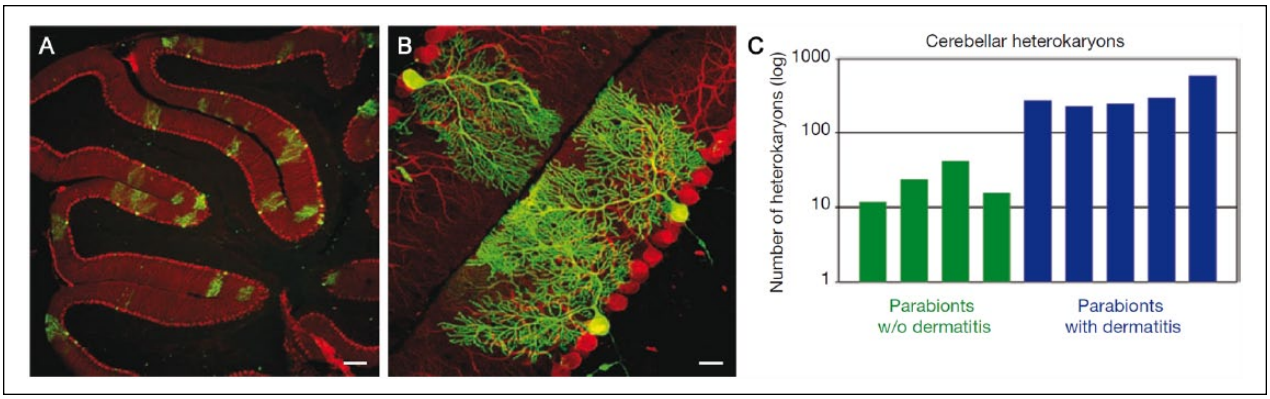

Figure 2. Brain damage and inflammation increase the integration of BMDC into the encephalon. (A, B) Cerebellar sagittal sections from a mouse with severe dermatitis and inflammation. Immunohistochemistry for GFP (bone marrow–derived elements; green) and calbindin D-28k (Purkinje cells; red) demonstrates that numerous heterokaryons have been formed in the cerebellum. (B) A confocal image of heterokaryons shows their normal morphology with GFP+ cell bodies, dendrites extending into the molecular layer, and an axon descending through the granular cell layer. (C) Quantification of cerebellar heterokaryons observed in parabionts with or without idiopathic ulcerative dermatitis. Scale bars are 100 μm (A) and 20 μm (B). Adapted by permission from Macmillan Publishers Ltd: Nature Cell Biology (Johansson and others), copyright (2008).

when premature transplantation is necessary because of the need for early treatment of a neurodegenerative process, such as during the perinatal stages (Espejel and others 2009; Recio and others 2011).

Finally, the main advantage of the use of chemical ablation versus irradiation is the lack of impairment to cell proliferation in neurogenic niches and other neuronal injuries (see above; Espejel and others 2009; Magrassi and others 2007). However, these treatments do not open the blood-brain barrier or facilitate restoration of the hematopoietic system (see above; Nygren and others 2008). It is clear that slower renovation of the hematopoietic system can also delay the arrival of BMDC in the brain, but there is some controversy about a possible opening of the blood-brain barrier. First, some findings support the idea that radiation increases the arrival of BMDC in the brain, chemical ablation, thus reducing integration (Wiersema and others 2007). By contrast, other authors have not reported differences between chemical and physical ablation of the bone marrow, thus radiation (and hence the opening of the blood-brain barrier deriving from this) not being necessary for such integration to occur (Espejel and others 2009; Johansson and others 2008; Magrassi and others 2007).

Genetic Ablation of Bone Marrow

The last possibility to ensure an empty niche for transplanting new bone marrow is to take advantage of genetically modified animals that lack this specific tissue (Fig. 1C). In this case, there is no real bone marrow ablation and hence no damage occurs to the host tissues. Indeed, in one of the first two works that reported the contribution of BMDC to the formation of neurons (Mezey and others 2000), PU.1 *knockout* mice were employed (Box 5). These animals are normal at birth, but require bone marrow transplantation to survive (Mezey and others 2000). There are also murine models of "partial bone marrow ablation," which are able to generate only certain types of blood cells (Nygren and others 2008). These models have also been employed as donors to detect the specific origin of bone marrow–derived neurons: stromal or hematopoietic (see below; Nygren and others 2008).

Box 5.
PU.1

PU.1 is a transcription factor that is only expressed in the cells of the hematopoietic lineage. Therefore, PU.1-null mice lack macrophages, neutrophils, mast cells, osteoclasts, and B and T cells, needing bone marrow transplantation.

No Ablation of Bone Marrow

As pointed out above, there are several methodologies for transplanting bone marrow SC or their derivatives that do not require bone marrow ablation. Thus, it is also possible to transplant bone marrow cells directly into the encephalic parenchyma or the cerebrospinal fluid (Bae and others 2007; Jones and others 2010; Kopen and others 1999), into blood vessels (without bone marrow ablation, attempting the direct the arrival of cells at the encephalon; Honmou and others 2012; Kemp and others 2011; Kocsis and Honmou 2012), or through the intranasal

pathway (Danielyan and others 2009) of rodents. The latter methodology, which has only been developed recently, seems to avoid the problems raised by the others, that is, damage to the brain parenchyma or the retention of transplanted cells in organs other than the brain (see above). The particularities of these transplantation methodologies deserve further explanation and are addressed in the "Transplant" section of this review (see below).

Another possibility that does not require any bone marrow ablation is *parabiosis*. This procedure consists of joining two mice in line from the olecranon to the knee in order for them to share their blood flows (Wright and others 2001). This model has also been employed in research in the CNS employing BMDC, although with contradictory results (Johansson and others 2008; Massengale and others 2005; Wagers and others 2002). The group of Johansson (2008) demonstrated that the BMDC from one mouse can become integrated in the encephalon of the other one, both being joined by parabiosis. However, previous works employing this same technique did not find any bone marrow–derived neurons (Massengale and others 2005; Wagers and others 2002), microglia being the only neural cell type derived from the bone marrow, and then in low numbers (Massengale and others 2005). In addition, the survival times used in these previous works were longer than those described by the group of Johansson (2008). It is possible that in the most recent work (Johansson and others 2008) some uncontrolled factors may have elicited a higher mobilization of hematopoietic SC from the bone marrow, as compared with the other two studies (Massengale and others 2005; Wagers and others 2002). More precisely, the group of Johansson (2008) found that idiopathic ulcerative dermatitis increased the fusion of hematopoietic SC with Purkinje cells to a noteworthy extent. It is possible that the animals joined by parabiosis and not diagnosed with dermatitis could suffer an attenuated manifestation of the illness. Thus, this uncontrolled manifestation could elicit some inflammation, increasing the number of fusion events in animals that were thought to be completely healthy.

In any case, direct brain injections as well as bone marrow ablation and transplantation are invasive methodologies. Moreover, although to a lesser extent intranasal transplants and parabiosis are also invasive, as foreign cells are being introduced in a recipient. Taking this into account, there is a final possibility for following up BMDC under strictly noninvasive conditions: the use of transgenic animals with specific labeling in their own bone marrow cells (Nern and others 2009). This methodology allows BMDC to be tracked in the body of a given animal without any kind of transplantation. Surprisingly, regarding this it has been reported that the behavior of BMDC could vary considerably from that previously demonstrated, at least by means of cell integration into the encephalon. For instance, Nern's group (2009) has reported that under these physiologic and noninvasive conditions BMDC do not establish stable heterokaryons with Purkinje neurons in mice, contrary to the main hypothesis (Álvarez-Dolado and others 2003; Díaz and others 2012b; Johansson and others 2008; Magrassi and others 2007; Recio and others 2011; and other laboratories), and the authors proposed a transitory fusion between both types of cells, this even being suitable for gene delivery or other therapies with BMDC.

Final Tips regarding Bone Marrow Ablation

Finally, after bone marrow ablation (which necessarily impairs the immune system) recipients should be provided with some conditions of sterility defined by current legislation. Accordingly, they should be confined in isolation cages, their food should be irradiated, and to avoid opportunistic infections, their drinking water should be controlled by at least one of the following procedures: sterilization, the addition of antibiotics (Chen and others 2011; Vallieres and Sawchenko 2003), or acidification (Álvarez-Dolado and others 2003).

Regarding rejection, bone marrow cells—especially stromal cells—have a low rejection rate (Tögel and Westenfelder 2007), and this phenomenon has not been reported in studies performing transplants only of bone marrow cells in mice (Díaz and others 2011; Díaz and others 2012a; Díaz and others 2012b; Eglitis and Mezey 1997; Priller and others 2001; Recio and others 2011; and other laboratories), but also in transplants from rats in mice (Johansson and others 2008).

Preparation of Donor Bone Marrow

In humans, it is relatively easy to harvest bone marrow SC to perform cell therapy (Mimeault and Batra 2006). However, when working with small animal models the donors must be sacrificed to obtain these SC after crushing their bones (often femurs, tibias, or hips) or flushing the bone marrow directly with culture medium. Therefore, and contrary to recipients, there is no special preparation required for the donor animals themselves. However, to a certain extent any modification of the donor bone marrow before transplantation into the recipient can be considered "a preparation." Accordingly, there exists the possibility of transplanting whole bone marrow—the most frequent technique—or fractionating it into its different cellular components: hematopoietic or stromal SC (Travlos 2006). This cell sorting, or at least the specific labeling of one variety of such cells, has led to the determination of the contribution of each cell type. Notwithstanding the complexity of the methodology, the results of these experiments are controversial regarding the specific cell type that contributes to neural elements.

Box 6.

Mesenchymal stem cells

A type of SC that have generally been obtained from the bone marrow stroma. Therefore, stromal SC are commonly called *mesenchymal*. However, mesenchymal SC are not exclusive to bone marrow but appear in nearly all organs, such as brain, spleen, liver, kidney, lung, bone marrow, muscle, thymus, and pancreas.

Sorting and Transplantation of Bone Marrow–Derived Stromal Cells

Stromal SC form a heterogenic group composed of different cell types, mesenchymal SC being the most abundant (Box 6; Tögel and Westenfelder 2007). These cells can differentiate into a broad variety of types of different tissues and have therefore been used in different cell therapies (Mimeault and Batra 2006; Tögel and Westenfelder 2007). Stromal/mesenchymal SC have usually been injected directly into the brain to study their features, showing excellent properties of migration, integration, and differentiation in both glial and neuronal networks, as well as neuroprotective effects without any neural differentiation in both rats and mice (Kopen and others 1999; Muñoz-Elías and others 2004). Therefore, such cells are also employed in promising cell therapies in rodents (Bae and others 2005; Jones and others 2010), also carried out by systemic infusion (Honmou and others 2012; Kemp and others 2011; Kocsis and Honmou 2012). Moreover, the therapeutic potential of BMDC seems to be due to mesenchymal SC exclusively, as a better beneficial effect of such mesenchymal SC has been reported in comparison with that of cells derived from whole murine bone marrow (without fractionating; Jones and others 2010). It should be noted that the therapeutic benefit of these local engraftments seems to be due to the release of neurotrophic factors that can elicit neuroprotection, because direct cell differentiation of mesenchymal SC toward neuronal elements was very scarce (in this case, fusion with Purkinje neurons; Jones and others 2010).

Sorting and Transplantation of Hematopoietic Stem Cells

In parallel to the potential of mesenchymal SC, it has been demonstrated in mice that a single hematopoietic SC can repopulate bone marrow and give rise *in vivo* to cells of different tissues, showing that this cell type is not only fated to form blood cells (Krause and others 2001). This latter work did not report any hematopoietic contribution to neural systems (Krause and others 2001), even though the first report to unveil the integration of BMDC into the encephalon had suggested—4 years previously—a hematopoietic origin of these BMDC (Eglitis and Mezey 1997). However, in this work the different bone marrow cell populations were not separated. Thus, the hematopoietic origin of bone marrow–derived neural cells could not be ensured. The plasticity potential of murine hematopoietic SC in brain was finally proved, although with low efficiency under physiological conditions (Wagers and others 2002), which could explain the previous lack of success in such detection (Krause and others 2001). In addition, this low efficiency may be blurred by transitory plasticity events, which are more difficult to detect than stable integrations in the nervous system (Nern and others 2009). Conversely, hematopoietic SC display a high rate of integration in the encephalon when tissue damage exists, and they have even been employed in cell therapy (Chen and others 2011; Johansson and others 2008). Also, the group of Johansson (2008) demonstrated that the bone marrow–derived cell type that fuses with Purkinje neurons is the hematopoietic lineage, at least in rats and mice. Finally, and more to the point, it has also been demonstrated that fusion in mice is due exclusively to the lymphoid lineage and not to the myeloid one (Nygren and others 2008).

In light of these data, the results of studies with hematopoietic SC are in direct disagreement with those employing mesenchymal cells. However, there are some differences in the way the cells were administered, and also in the experimental model used, that could account for this controversy. On the one hand, in most studies employing mesenchymal SC in rodents, these cells were injected directly into the encephalon (Bae and others 2005; Bae and others 2007; Jones and others 2010; Kopen and others 1999; Muñoz-Elías and others 2004), bone marrow ablation and subsequent reconstitution not being necessary in these cases. An exception in the way of administration is the work reported by the group of Kemp (2011), who performed systemic injections of human mesenchymal SC in mice, also without bone marrow ablation. By contrast, in experiments employing hematopoietic cells (and also whole bone marrow) initial bone marrow ablation and subsequent systemic cell infusion were performed (Chen and others 2011; Eglitis and Mezey 1997; Krause and others 2001). Thus, although both types of bone marrow SC have the capacity to give rise to neural elements due to their plasticity (Tögel and Westenfelder 2007), mesenchymal SC seem to be more suitable for brain integration after direct engraftments or for neuroprotection (Jones and others 2010), whereas we have reason to think that hematopoietic SC are more appropriate for integration after systemic transplants, especially after bone marrow niche

replenishment. The point common to both mesenchymal and hematopoietic SC is that neural damage (especially with inflammation) increases their integration into the encephalon (Fig. 2; Bae and others 2005; Johansson and others 2008).

Transplantation

Above we have described different possibilities for the ablation of bone marrow before transplantation, also taking into account the option of no ablation. Moreover, the process of transplantation itself is also quite variable, with diverse types of parameters (apart from the type of transplanted cells, see above).

Site of Injection

We have previously mentioned that engraftments can be performed directly into the encephalon or by means of other types of injections (systemic, intraperitoneal, or intrahepatic). It should be recalled that the latter methodologies are aimed at replenishing the recipient's bone marrow when it has been removed previously. However, systemic injections can also be performed without bone marrow ablation in order to deliver cells to the brain through blood vessels (see above).

The first option (encephalic injections) is performed with a stereotaxic instrument, injecting the cells directly into the desired region of the encephalon by means of pressure-driven capillaries (Bae and others 2005; Jones and others 2010; Kopen and others 1999; Moore and others 2005). Regarding systemic injections, there are also different possibilities in rodents, the intravenous type being the most frequent in comparison with intra-arterial or intraperitoneal ones. These injections are often made in the tail vein (Corti and others 2002; Díaz and others 2012a; Díaz and others 2012b; Eglitis and Mezey 1997; Magrassi and others 2007; Recio and others 2011; and other laboratories), but they can also be performed in the retro-orbital plexus (Massengale and others 2005), in the maxillofacial vein (Piquer-Gil and others 2009), or through the intracardiac route (Rodríguez and others 2007), the latter having the drawback of needing to perform surgery with the animals under anesthesia, with a mortality of 2%. Other common methods are intraperitoneal (Álvarez-Dolado and others 2003; Mezey and others 2000) or intrahepatic transplantation (Espejel and others 2009; Massengale and others 2005). The implementation of these latter two techniques is easier than intravenous transplantation and hence they are commonly employed for injections in newborns and even in in utero transplantation (Nygren and others 2008).

Number of Transplanted Cells

It has been demonstrated that a single cell can repopulate the hematopoietic system and give rise to other cells in different tissues (Krause and others 2001). However, the number of transplanted cells is usually much higher, with the aim of ensuring their integration. In experiments performed directly in the encephalon or in intranasal administration, the number of transplanted cells rarely surpasses one million (Table 3). In systemic injections, the number of transplanted cells is much more elevated (several million; Table 3). Evidently, the encephalic parenchyma has a limited space (especially in laboratory rodents) in which cell transplantation can be performed and hence the volume of the transplant should be smaller than in systemic injections (likewise the droplet of cell suspension employed in intranasal transplants; see below).

Efficiency of Transplantation

The efficiency of these techniques is determined in recipient animals by the degree of chimerism of the donor cells in the host tissues, especially with bone marrow reconstitution. This is usually referred to as the percentage of donor-derived nucleated cells in the recipient's blood. Nevertheless, this percentage can be also determined in the spleen or the liver (Mezey and others 2000). Regarding this parameter, intravenous transplants—in all of the above-mentioned possibilities—of whole bone marrow (without fractionating) seem to be very efficient, with percentages of blood cell chimerism of 50% to 85% (Krause and others 2001; Priller and others 2001) or even higher, up to 99% (Johansson and others 2008), reaching maximum levels of GFP-positive blood cells comparable to those from donors (Díaz and others 2011). However, if only a fraction of the bone marrow is transplanted—or only a single cell—the average efficiency is lower (although very rarely levels of up to 85%, or even 98% are possible: Krause and others 2001; Massengale and others 2005; Nygren and others 2008). This average lower efficiency of transplanting a fraction of the bone marrow in comparison with whole bone marrow transplantations may be due to a slower reconstitution of the bone marrow niche. It is also important to keep in mind that radiation can also accelerate or facilitate the reconstitution of new bone marrow (Nygren and others 2008). Regarding the other types of transplantation (intraperitoneal and intrahepatic), blood chimerism usually ranges between 50% and 80%, but is much more variable, without the predominance of higher percentages and also with much lower values (Massengale and others 2005). These lower values could also be explained in terms of the chemical ablation of the bone marrow (no facilitation by radiation; Nygren and others 2008), the injection of cells

Table 3. Number and Type of Transplanted Cells.

Intracranial	
50,000–100,000	Kopen and others 1999 (stromal); Muñoz-Elías and others 2004 (stromal); Moore and others 2005 (hematopoietic)
500,000	Bae and others 2005 (mesenchymal); Jones and others 2010 (mesenchymal)
1 million	Bae and others 2007 (mesenchymal)
Intranasal	
100,000	Fransson and others 2012 (T-cells)
300,000	Danielyan and others 2009 (mesenchymal); Danielyan and others 2011 (mesenchymal); Reitz and others 2012 (neural progenitors)
500,000	van Velthoven and others 2010 (mesenchymal)
Systemic	
≤1 million	Chen and others 2011 (whole bone marrow enriched with hematopoietic cells); Kemp and others 2011 (mesenchymal)
2–3 million	Eglitis and Mezey 1997 (hematopoietic)
5–8 million	Priller and others 2001 (whole bone marrow); Vallieres and Sawchenko 2003 (whole bone marrow); Rodríguez and others 2007 (whole bone marrow); Nygren and others 2008 (whole bone marrow); Johansson and others 2008 (whole bone marrow); Díaz and others 2011 (whole bone marrow); Díaz and others 2012a (whole bone marrow); Díaz and others 2012b (whole bone marrow); Recio and others 2011 (whole bone marrow)
10 million	Corti and others 2002 (whole bone marrow); Wiersema and others 2007 (whole bone marrow); Weimann and others 2003b (whole bone marrow); Espejel and others 2009 (whole bone marrow)
10–20 million	Álvarez-Dolado and others 2003 (whole bone marrow)
30 million	Corti and others 2004 (whole bone marrow)

outside the blood stream (and the subsequent barriers for the bone marrow to be reached), or incomplete bone marrow ablation in newborns (because of their high rates of cell proliferation).

Transplants in Brain Parenchyma

In contrast to the situation in pure basic research addressing cell integration in the encephalon, this methodology is often employed in models of neurodegeneration or brain injury. In this case, the migratory properties of the grafted cells are very important, as the recipients usually receive a single injection close to the damaged region (Bae and others 2007; Jones and others 2010). The use of this methodology is influenced by different factors. For instance, in both mice and rats the site of cell injection affects cell migration, the cells becoming disseminated farther if they are injected into the brain ventricles than into the parenchyma (Jones and others 2010; Kopen and others 1999; Moore and others 2005; Muñoz-Elías and others 2004). The age of the recipient also affects the quality of the engraftments. Hence, transplants in newborns, whose brain is still developing, increase the integration of grafted cells in the whole encephalon, also giving rise to cell types different from those derived from transplants accomplished with other methodologies. This is the case of bone marrow–derived astrocytes, which were only detected in the first report of the contribution of BMDC to the CNS of mice (Eglitis and Mezey 1997) and in transplants in newborns (Kopen and others 1999), but not in other studies performed on adult animals (Díaz and others 2011; Recio and others 2011; Wehner and others 2003; and other laboratories). Moreover, bone marrow cells grafted into the adult brain can also maintain their hematopoietic phenotype (Moore and others 2005), supporting the hypothesis of lower differentiation rates in adult animals than in newborns. Finally, encephalic damage can also increase the integration of BMDC transplanted directly into the brain parenchyma (Bae and others 2005), in consonance with the results of methodologies that replace the bone marrow of the recipient (see above).

Intranasal Transplants

Developed for first time by Danielyan and others (2009), this method involves the release of 2- to 6-μL droplets of a cell suspension into the nostrils of both mice and rats, whether awake (Reitz and others 2012; van Velthoven and others 2010) or under anesthesia (Danielyan and others 2009; Wei and others 2013). This simple method shuttles the transplanted cells to the encephalon, avoiding invasive procedures such as injection into the brain (with the subsequent damage to the parenchyma) or ablation of the recipient's bone marrow (Danielyan and others 2009; Jiang and others 2011). Moreover, the proximity of the site of transplant to the brain would also hinder the retention of transplanted cells in other organs, as may occur with systemic

injections (Danielyan and others 2009). Another advantage of this methodology is the short time that the transplanted cells take to reach the encephalon. Thus, 1 hour after transplant cells can be detected mainly in the olfactory bulb, and also in much more caudal regions such as the thalamus, the cortex, the hippocampus, or even the cerebellum (Danielyan and others 2009). In addition, the efficiency of this method can be enhanced by the application of hyaluronidase prior to transplantation (Danielyan and others 2009; Wei and others 2013). Regarding the specific route of transport from the nostrils to the brain, several hypotheses have been advanced, although they lack confirmation (Danielyan and others 2009).

The survival of transplanted cells after intranasal administration has been demonstrated for at least up to 6 months (Danielyan and others 2011), allowing their application in therapeutic practice. Moreover, these intranasally transplanted cells are also attracted by brain damage (Danielyan and others 2011; Fransson and others 2012; van Velthoven and others 2010; Wei and others 2013) and tumors (Reitz and others 2012). By contrast, cells transplanted intranasally are usually mesenchymal SC, but there is no conclusive evidence of their differentiation into neural elements, apart from the appearance of some ambiguous, neurite-like cell processes (Danielyan and others 2011). Accordingly, their benefits are only due to the release of neuroprotective or neuroactive substances. Interestingly, when transplanted cells are more differentiated, for example, T regulatory cells, integration in the encephalon is stronger, neural elements such as thalamic, cortical, or even Purkinje neurons appearing (Fransson and others 2012). Although such integration remains to be fully understood, it seems to support the hypothesis of a hematopoietic origin of bone marrow–derived neural elements (see above).

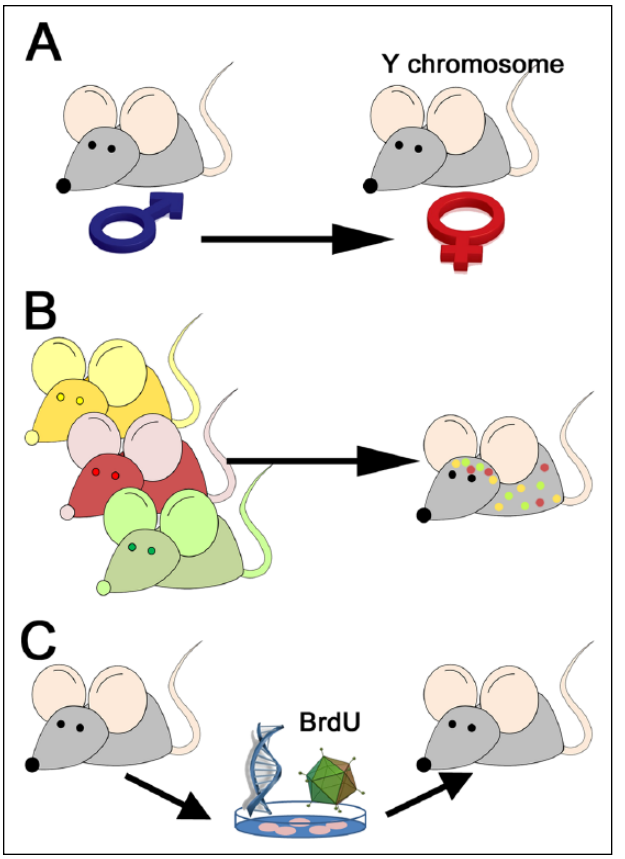

Figure 3. Different methodologies for tracking the cells derived from the bone marrow transplanted. Transplants can be performed from a male into a female, the cells carrying a Y chromosome being the donor-derived elements (A). The most common method for cell tracking is to inject bone marrow cells from an animal expressing green, yellow or red fluorescent protein in a nonfluorescent animal (B), thus allowing their detection by their specific fluorescent labeling. Finally, cells extracted from a donor can receive a pulse of BrdU, or can also be labeled or modified genetically, viral transfection being a common technique for this purpose (C).

Tracking and Visualization of Transplant-Derived Cells

As pointed out earlier, the labeling of transplanted bone marrow cells can be considered part of the preparation of the donor. However, the aim of this labeling is to detect such cells and their progeny in the encephalon (or other organs) of the recipient. This therefore requires attention in a separate section.

Y Chromosome

To detect cells derived from the transplant, it is necessary for these cells to have some special feature that makes them distinguishable from host tissues. In the first report that demonstrated the integration of BMDC in the encephalon (Eglitis and Mezey 1997), the strategy employed was to transplant bone marrow cells from a male mouse donor to a female (Fig. 3A). Thus, donor-derived cells were detected by *in situ* hybridization of the Y chromosome (Eglitis and Mezey 1997). This methodology, also used by other groups (Kale and others 2003), ensures the detection of BMDC as all the donor (male)-derived cells must have the Y chromosome. In fact, this technique has also been used to demonstrate the arrival of BMDC at the encephalon in humans (Mezey and others 2003; Weimann and others 2003a), employing samples from women that had suffered leukemia and had been transplanted with bone marrow derived from male donors. However, the limitation to this methodology is the actual characterization of BMDC, as the combination of in situ hybridization and immunohistochemistry is a somewhat involved technique (Eglitis and Mezey 1997).

Fluorescent Markers

The methodology most widely applied to detect cells derived from transplantation is the use of fluorescent markers for BMDC (Fig. 3B). Usually, the donors are transgenic animals (both mice and rats) that constitutively express fluorescent proteins, the green fluorescent protein (GFP) being the most frequent (Brazelton and others 2000; Díaz and others 2011; Díaz and others 2012b; Johansson and others 2008; Recio and others 2011; Weimann and others 2003b; and other laboratories). Donors can also express other fluorescent proteins, such as the red (RFP; Bae and others 2005; Bae and others 2007) or the yellow ones (YFP; Corti and others 2004), which are colors of special interest if the recipients also express the GFP in certain cell types (Bae and others 2005; Bae and others 2007). In addition, this system allows easy characterization of BMDC by immunofluorescence with other fluorescent colors, and it also enables verification of the incorporation of the transplant into the host body by means of flow cytometry of the peripheral blood. The negative side of this method is that although fluorescent proteins are under the control of a constitutive promoter, not all donor cells express them (Díaz and others 2011), probably through gene silencing (Long and Rossi 2009). Sometimes, donors are genetically modified to express GFP (or other fluorescent proteins) in specific cell types such as the hematopoietic (Nern and others 2009; Wagers and others 2002) or lymphoid lineages (Nygren and others 2008).

Donor cells can also be labeled outside the donor body once they have been extracted by transfection with viruses that encode GFP (Fig. 3C; Priller and others 2001). This method is carried out after the extraction of donor cells and before transplantation, but allows certain extra modifications of these cells, such as *knock in* for certain genes to deliver them to the recipient if the animal is defective for a gene, thereby combining cell and gene therapies (Chen and others 2011).

Xenogenic Markers

If transplants are performed between different species—xenotransplants—it is also possible to follow the destination of the transplant-derived cells by detecting specific proteins of the donor species, that is, from humans in mice (Kemp and others 2011) or from rats in mice (Johansson and others 2008). More than as a method of detection, this technique has been employed to analyze certain properties of transplanted cells, that is, whether human BMDC have the capacity to become integrated into host tissues, in this case an animal model being necessary (Kemp and others 2011; Reitz and others 2012), or to determine the capacity of genetic expression of donor-derived cells (Johansson and others 2008). In any case, these xenotransplants of bone marrow SC may develop more graft-versus-host disease than transplants performed between animals of the same species (Chu and Gress 2008).

Other Tracking Methods

Bone marrow SC are proliferating cells and hence once extracted they can also be labeled with a pulse of BrdU before their transplantation (Fig. 3C; Kopen and others 1999; Muñoz-Elías and others 2004). However, this labeling would not be useful for the long-term tracking of BMDC because of the progressive dilution of BrdU in successive cell divisions.

Moreover, donors and recipients can also be modified genetically in order to detect the process of integration of BMDC into host tissue. This is the case of mice with the Cre-*LacZ* system (Fig. 4), which was employed for the first time for this purpose by Álvarez-Dolado and others (2003) and then by other researchers (Espejel and others 2009; Nern and others 2009; Piquer-Gil and others 2009; Recio and others 2011).

Finally, there is also the possibility of visualizing transplanted cells *in vivo* by employing positron emission tomography (PET) or real-time magnetic resonance imaging (MRI; Doudet and others 2004). PET allows cells labeled with isotopes to be tracked (Doudet and others 2004), whereas MRI is suitable for fluorescence-labeled cells (Fiandaca and others 2009). However, both techniques are only employed after engraftments performed directly in the brain parenchyma, as large numbers of clustered labeled cells are necessary if they are to be detected (Doudet and others 2004; Fiandaca and others 2009; Thompson and others 2005). Thus, it is not possible to detect cells derived from transplanted bone marrow, which are integrated into the brain either one by one or in discrete, disseminated populations.

From the Bench to the Bedside: Future Human Application and Current Clinical Trials

In this review, different parameters for research with BMDC in the CNS have been summarized. Clearly, the final goal of research is not only to determine the properties of integration and migration of the transplanted cells but also their therapeutic potential. In this sense, BMDC are capable of transdifferentiating into neural elements as well as fusing with specific neurons, raising the possibility for substitutive and regenerative therapies or for gene delivery through fusion. However, the efficacy of these processes is low (Wagers and others 2002) and only Purkinje neurons fuse with BMDC (Álvarez-Dolado and others 2003). Thus, much research remains to be done before the BMDC

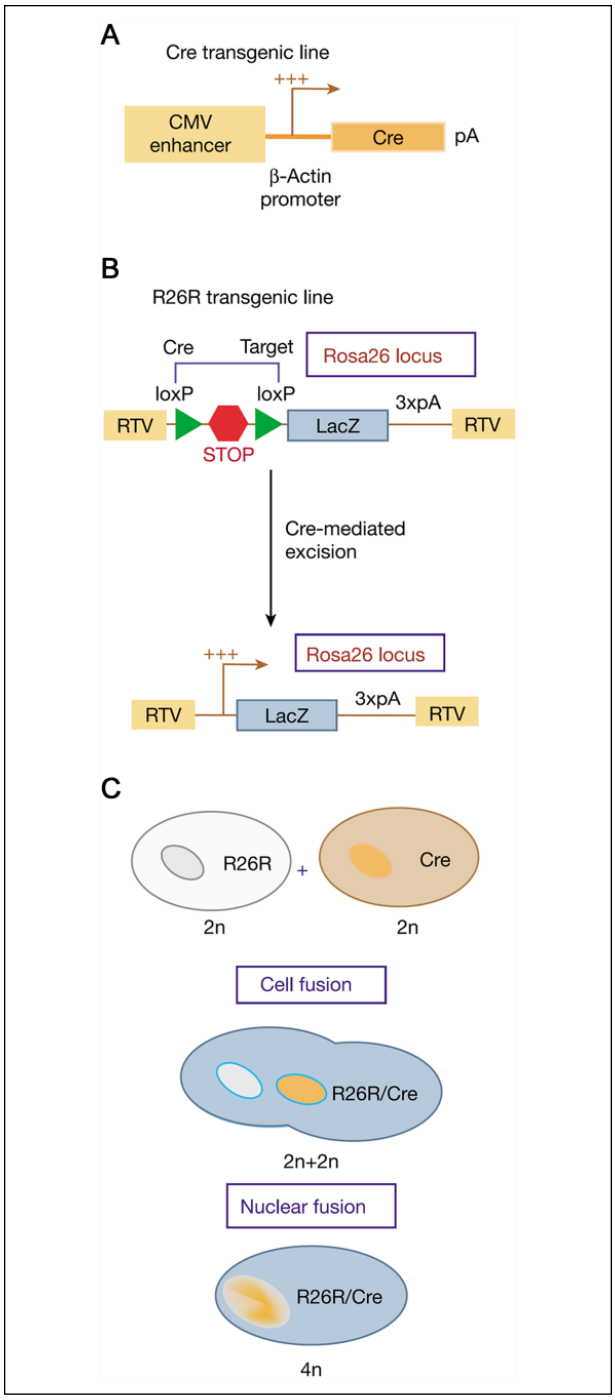

Figure 4. Method for detecting cell fusion events. (A, B) Schematic representation of the transgenes expressed by the mouse lines used for this purpose. (C) When a cell expressing Cre recombinase (A) fuses with a cell bearing the *LacZ* reporter transgene (B), the stop cassette located between two loxP sites (floxed) is excised and the *LacZ* reporter is expressed in the fused cell. *LacZ* expression can be detected by the generation of a blue precipitate after X-gal staining or by immunohistochemistry against the translated protein (β-galactosidase). RTV, Integration retroviral sequence (LTR). Adapted by permission from Macmillan Publishers Ltd: Nature (Álvarez-Dolado and others), copyright (2003).

properties of transdifferentiation and fusion can be suitably employed in clinical practice. In addition, much of the research reviewed here involves highly invasive methods (i.e., involving encephalic damage, high doses of radiation, intrauterine administration, etc.), which for ethical and operational reasons raise concerns in humans. Accordingly, ongoing clinical trials concerning the use of BMDC in neural diseases take advantage of the immunomodulatory, neurotrophic and neuroprotective properties of these cells.

Currently, there are many clinical trials that can be consulted readily at the www.ClinicalTrials.gov website. These investigations employ unfractionated bone marrow cell populations, stromal (mesenchymal) or hematopoietic SC, or even more restricted cell populations. In addition, the routes of administration are open to many different possibilities, mainly systemic (usually into the carotid artery), intrathecal, into the ependymal canal, and even into the neural parenchyma (i.e., spinal cord). The variety of techniques employed in basic/translational research is also reflected in clinical practice. Moreover, the vast majority of clinical trials are in phase I or II, and are therefore related to the safety of the treatment and possible therapeutic benefits, but do not consider the use of systematic use. In any case, a large number of different neural diseases has been studied in clinical trials with BMDC. Some of these studies have begun to yield promising results, as reflected in scientific publications: stroke (Moniche and others 2012), Parkinson's disease (Venkataramana and others 2010), cerebral palsy (Giordano and others 2014), multiple sclerosis (Connick and others 2011; Karussis and others 2010), epilepsy, cancers of the CNS (Kreissman and others 2013), traumatic brain injuries (Sharma and others 2012), autism, spinal cord injury (Geffner and others 2008), amyotrophic lateral sclerosis (Blanquer and others 2012; Karussis and others 2010), retinitis pigmentosa (Siqueira and others 2013), cerebellar ataxia, and Huntington's chorea.

Final Remarks

We have summarized the multiple parameters that should be taken into account in order to employ bone marrow SC for research in the CNS. Each factor or technique has its *pros* and its *cons*, and can generate different secondary effects or conditions that can also influence the integration of BMDC into the encephalic parenchyma. Depending on the goals and conditions, researchers should choose the methodologies that best match the requirements of a given experiment in order to perform it with the fewest secondary effects that might interfere in the results. In addition, analysis of these effects, beyond those of transplantation itself, should also clarify unexpected results. Hopefully taking these parameters into account, research can move forward in the promising field of cell therapy in the CNS.

Acknowledgments

The authors express their gratitude to N. Skinner for revising the English version of the article and to L. Muñoz de la Pascua for the support referring animal care.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: the Ministerio de Investigación y Ciencia (BFU2010-18284), the Junta de Castilla y León, and the Centre for Regenerative Medicine and Cell Therapy of Castilla y León.

References

Altman J. 1969. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–57.

Álvarez-Dolado M. 2007. Cell fusion: biological perspectives and potential for regenerative medicine. Front Biosci 12: 1–12.

Álvarez-Dolado M, Pardal R, García-Verdugo JM, Fike JR, Lee HO, Pfeffer K, and others. 2003. Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 425:968–73.

Bae JS, Furuya S, Shinoda Y, Endo S, Schuchman EH, Hirabayashi Y, and others. 2005. Neurodegeneration augments the ability of bone marrow-derived mesenchymal stem cells to fuse with Purkinje neurons in Niemann-Pick type C mice. Hum Gene Ther 16:1006–11.

Bae JS, Han HS, Youn DH, Carter JE, Modo M, Schuchman EH, and others. 2007. Bone marrow-derived mesenchymal stem cells promote neuronal networks with functional synaptic transmission after transplantation into mice with neurodegeneration. Stem Cells 25:1307–16.

Balentova S, Racekova E, Martoncikova M, Misurova E. 2006. Cell proliferation in the adult rat rostral migratory stream following exposure to gamma irradiation. Cell Mol Neurobiol 26:1131–9.

Balentova S, Racekova E, Misurova E. 2007. Effect of low-dose irradiation on proliferation dynamics in the rostral migratory stream of adult rats. Folia Biol (Praha) 53:74–8.

Becker AJ, McCulloch EA, Till JE. 1963. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. Nature 197:452–4.

Blanquer M, Moraleda JM, Iniesta F, Gómez-Espuch J, Meca-Lallana J, Villaverde R, and others. 2012. Neurotrophic bone marrow cellular nests prevent spinal motoneuron degeneration in amyotrophic lateral sclerosis patients: a pilot safety study. Stem Cells 30:1277–85.

Brazelton TR, Rossi FM, Keshet GI, Blau HM. 2000. From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290:1775–9.

Calió ML, Marinho DS, Ko GM, Ribeiro RR, Carbonel AF, Oyama LM, and others. 2014. Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model. Free Radic Biol Med 70C:141–54.

Casper J, Knauf W, Kiefer T, Wolff D, Steiner B, Hammer U, and others. 2004. Treosulfan and fludarabine: a new toxicity-reduced conditioning regimen for allogeneic hematopoietic stem cell transplantation. Blood 103:725–31.

Chen KA, Cruz PE, Lanuto DJ, Flotte TR, Borchelt DR, Srivastava A, and others. 2011. Cellular fusion for gene delivery to SCA1 affected Purkinje neurons. Mol Cell Neurosci 47:61–70.

Chu YW, Gress RE. 2008. Murine models of chronic graft-versus-host disease: insights and unresolved issues. Biol Blood Marrow Transplant 14:365–78.

Connick P, Kolappan M, Patani R, Scott MA, Crawley C, He XL, and others. 2011. The mesenchymal stem cells in multiple sclerosis (MSCIMS) trial protocol and baseline cohort characteristics: an open-label pre-test: post-test study with blinded outcome assessments. Trials 12:62.

Corti S, Locatelli F, Donadoni C, Guglieri M, Papadimitriou D, Strazzer S, and others. 2004. Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues. Brain 127:2518–32.

Corti S, Locatelli F, Strazzer S, Salani S, Del Bo R, Soligo D, and others. 2002. Modulated generation of neuronal cells from bone marrow by expansion and mobilization of circulating stem cells with in vivo cytokine treatment. Exp Neurol 177:443–52.

Danielyan L, Schafer R, von Ameln-Mayerhofer A, Bernhard F, Verleysdonk S, Buadze M, and others. 2011. Therapeutic efficacy of intranasally delivered mesenchymal stem cells in a rat model of Parkinson disease. Rejuvenation Res 14:3–16.

Danielyan L, Schafer R, von Ameln-Mayerhofer A, Buadze M, Geisler J, Klopfer T, and others. 2009. Intranasal delivery of cells to the brain. Eur J Cell Biol 88:315–24.

de Jong R, Houtgraaf JH, Samiei S, Boersma E, Duckers HJ. 2014. Intracoronary stem cell infusion after acute myocardial infarction: a meta-analysis and update on clinical trials. Circ Cardiovasc Interv 7:156–67.

Díaz D, Lepousez G, Gheusi G, Alonso JR, Lledo PM, Weruaga E. 2012a. Bone marrow cell transplantation restores olfaction in the degenerated olfactory bulb. J Neurosci 32: 9053–8.

Díaz D, Recio JS, Baltanás FC, Gómez C, Weruaga E, Alonso JR. 2011. Long-lasting changes in the anatomy of the olfactory bulb after ionizing irradiation and bone marrow transplantation. Neuroscience 173:190–205.

Díaz D, Recio JS, Weruaga E, Alonso JR. 2012b. Mild cerebellar neurodegeneration of aged heterozygous PCD mice increases cell fusion of Purkinje and bone marrow-derived cells. Cell Transplant 21:1595–602.

Doetsch F, García-Verdugo JM, Álvarez-Buylla A. 1997. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 17:5046–61.

Doudet DJ, Cornfeldt ML, Honey CR, Schweikert AW, Allen RC. 2004. PET imaging of implanted human retinal pigment epithelial cells in the MPTP-induced primate model of Parkinson's disease. Exp Neurol 189:361–8.

Down JD, Boudewijn A, Dillingh JH, Fox BW, Ploemacher RE. 1994. Relationships between ablation of distinct haematopoietic cell subsets and the development of donor bone marrow engraftment following recipient pretreatment with different alkylating drugs. Br J Cancer 70:611–6.

Eglitis MA, Mezey E. 1997. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci U S A 94:4080–5.

Espejel S, Romero R, Álvarez-Buylla A. 2009. Radiation damage increases Purkinje neuron heterokaryons in neonatal cerebellum. Ann Neurol 66:100–9.

Ferrari G, Cusella-De AG, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, and others. 1998. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 279: 1528–30.

Fiandaca MS, Varenika V, Eberling J, McKnight T, Bringas J, Pivirotto P, and others. 2009. Real-time MR imaging of adeno-associated viral vector delivery to the primate brain. Neuroimage 47(Suppl 2):T27–T35.

Fransson M, Piras E, Burman J, Nilsson B, Essand M, Lu B, and others. 2012. CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery. J Neuroinflammation 9:112.

Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. 1968. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation 6:230–47.

Fuchs E, Tumbar T, Guasch G. 2004. Socializing with the neighbors: stem cells and their niche. Cell 116:769–78.

Geffner LF, Santacruz P, Izurieta M, Flor L, Maldonado B, Auad AH, and others. 2008. Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. Cell Transplant 17:1277–93.

Giordano R, Canesi M, Isalberti M, Isaias IU, Montemurro T, Vigano M, and others. 2014. Autologous mesenchymal stem cell therapy for progressive supranuclear palsy: translation into a phase I controlled, randomized clinical study. J Transl Med 12:14.

Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. 1996. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 183:1797–806.

Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, and others. 1999. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401:390–4.

Hellstrom NA, Bjork-Eriksson T, Blomgren K, Kuhn HG. 2009. Differential recovery of neural stem cells in the subventricular zone and dentate gyrus after ionizing radiation. Stem Cells 27:634–41

Henning RJ. 2013. Stem cells for cardiac repair: problems and possibilities. Future Cardiol 9:875–84.

Herrmann RP, Sturm MJ. 2014. Adult human mesenchymal stromal cells and the treatment of graft versus host disease. Stem Cells Cloning 7:45–52.

Honmou O, Onodera R, Sasaki M, Waxman SG, Kocsis JD. 2012. Mesenchymal stem cells: therapeutic outlook for stroke. Trends Mol Med 18:292–7.

Ito H. 2014. Clinical considerations of regenerative medicine in osteoporosis. Curr Osteoporos Rep 12:230–8.

Jeong CH, Kim SM, Lim JY, Ryu CH, Jun JA, Jeun SS. 2014. Mesenchymal stem cells expressing brain-derived neurotrophic factor enhance endogenous neurogenesis in an ischemic stroke model. Biomed Res Int 2014:129145.

Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, and others. 2002. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418:41–9.

Jiang Y, Zhu J, Xu G, Liu X. 2011. Intranasal delivery of stem cells to the brain. Expert Opin Drug Deliv 8:623–32.

Johansson CB, Youssef S, Koleckar K, Holbrook C, Doyonnas R, Corbel SY, and others. 2008. Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation. Nat Cell Biol 10:575–83.

Jones J, Jaramillo-Merchan J, Bueno C, Pastor D, Viso-Leon M, Martinez S. 2010. Mesenchymal stem cells rescue Purkinje cells and improve motor functions in a mouse model of cerebellar ataxia. Neurobiol Dis 40:415–23.

Kale S, Karihaloo A, Clark PR, Kashgarian M, Krause DS, Cantley LG. 2003. Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. J Clin Invest 112:42–9.

Kanno H. 2013. Regenerative therapy for neuronal diseases with transplantation of somatic stem cells. World J Stem Cells 5:163–71.

Karussis D, Karageorgiou C, Vaknin-Dembinsky A, Gowda-Kurkalli B, Gomori JM, Kassis I, and others. 2010. Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis. Arch Neurol 67:1187–94.

Kemp K, Gordon D, Wraith DC, Mallam E, Hartfield E, Uney J, and others. 2011. Fusion between human mesenchymal stem cells and rodent cerebellar Purkinje cells. Neuropathol Appl Neurobiol 37:166–78.

Kikuchi H, Yagi H, Hasegawa H, Ishii Y, Okabayashi K, Tsuruta M, and others. 2014. Therapeutic potential of transgenic mesenchymal stem cells engineered to mediate anti-high mobility group box 1 activity: targeting of colon cancer. J Surg Res 190:134–43.

Kocsis JD, Honmou O. 2012. Bone marrow stem cells in experimental stroke. Prog Brain Res 201:79–98.

Kopen GC, Prockop DJ, Phinney DG. 1999. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 96: 10711–6.

Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, and others. 2001. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105:369–77.

Kreissman SG, Seeger RC, Matthay KK, London WB, Sposto R, Grupp SA, and others. 2013. Purged versus non-purged peripheral blood stem-cell transplantation for high-risk neuroblastoma (COG A3973): a randomised phase 3 trial. Lancet Oncol 14:999–1008.

Li L, Xie T. 2005. Stem cell niche: structure and function. Annu Rev Cell Dev Biol 21:605–31.

Li ZY, Wang CQ, Lu G, Pan XY, Xu KL. 2014. Effects of bone marrow mesenchymal stem cells on hematopoietic recovery and acute graft-versus-host disease in murine allogeneic umbilical cord blood transplantation model. Cell Biochem Biophys. Apr 3 [Epub ahead of print]

Locatelli F, Pession A, Bonetti F, Maserati E, Prete L, Pedrazzoli P, and others. 1994. Busulfan, cyclophosphamide and melphalan as conditioning regimen for bone marrow transplantation in children with myelodysplastic syndromes. Leukemia 8:844–9.

Long MA, Rossi FM. 2009. Silencing inhibits Cre-mediated recombination of the Z/AP and Z/EG reporters in adult cells. PLoS One 4:e5435.

Losordo DW, Vaughan DE. 2014. Going mobile: enhanced recovery from myocardial infarction via stem cell mobilization and homing for tissue repair. J Am Coll Cardiol 63:2873–4.

Magrassi L, Grimaldi P, Ibatici A, Corselli M, Ciardelli L, Castello S, and others. 2007. Induction and survival of binucleated Purkinje neurons by selective damage and aging. J Neurosci 27:9885–92.

Manda K, Ueno M, Anzai K. 2009. Cranial irradiation-induced inhibition of neurogenesis in hippocampal dentate gyrus of adult mice: attenuation by melatonin pretreatment. J Pineal Res 46:71–8.

Massengale M, Wagers AJ, Vogel H, Weissman IL. 2005. Hematopoietic cells maintain hematopoietic fates upon entering the brain. J Exp Med 201:1579–89.

Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. 2000. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 290:1779–82.

Mezey E, Key S, Vogelsang G, Szalayova I, Lange GD, Crain B. 2003. Transplanted bone marrow generates new neurons in human brains. Proc Natl Acad Sci U S A 100:1364–9.

Mimeault M, Batra SK. 2006. Concise review: recent advances on the significance of stem cells in tissue regeneration and cancer therapies. Stem Cells 24:2319–45.

Mizumatsu S, Monje ML, Morhardt DR, Rola R, Palmer TD, Fike JR. 2003. Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. Cancer Res 63:4021–7.

Moniche F, González A, González-Marcos JR, Carmona M, Pinero P, Espigado I, and others. 2012. Intra-arterial bone marrow mononuclear cells in ischemic stroke: a pilot clinical trial. Stroke 43:2242–4.

Monje ML, Mizumatsu S, Fike JR, Palmer TD. 2002. Irradiation induces neural precursor-cell dysfunction. Nat Med 8:955–62.

Moore BE, Colvin GA, Dooner MS, Quesenberry PJ. 2005. Lineage-negative bone marrow cells travel bidirectionally in the olfactory migratory stream but maintain hematopoietic phenotype. J Cell Physiol 202:147–52.

Muñoz-Elías G, Marcus AJ, Coyne TM, Woodbury D, Black IB. 2004. Adult bone marrow stromal cells in the embryonic brain: engraftment, migration, differentiation, and long-term survival. J Neurosci 24:4585–95.

Nern C, Wolff I, Macas J, von Rrandow J, Scharenberg C, Priller J, and others. 2009. Fusion of hematopoietic cells with Purkinje neurons does not lead to stable heterokaryon formation under noninvasive conditions. J Neurosci 29:3799–807.

Nygren JM, Liuba K, Breitbach M, Stott S, Thoren L, Roell W, and others. 2008. Myeloid and lymphoid contribution to non-haematopoietic lineages through irradiation-induced heterotypic cell fusion. Nat Cell Biol 10:584–92.

Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, and others. 1999. Bone marrow as a potential source of hepatic oval cells. Science 284:1168–70.

Piquer-Gil M, García-Verdugo JM, Zipancic I, Sánchez MJ, Álvarez-Dolado M. 2009. Cell fusion contributes to pericyte formation after stroke. J Cereb Blood Flow Metab 29:480–5.

Ploemacher RE, Johnson KW, Rombouts EJ, Etienne K, Westerhof GR, Baumgart J, and others. 2004. Addition of treosulfan to a nonmyeloablative conditioning regimen results in enhanced chimerism and immunologic tolerance in an experimental allogeneic bone marrow transplant model. Biol Blood Marrow Transplant 10:236–45.

Porat Y, Assa-Kunik E, Belkin M, Krakovsky M, Lamensdorf I, Duvdevani R, and others. 2014. A novel potential therapy for vascular diseases: blood-derived stem/progenitor cells specifically activated by dendritic cells. Diabetes Metab Res Rev. Mar 18 [Epub ahead of print]

Priller J, Persons DA, Klett FF, Kempermann G, Kreutzberg GW, Dirnagl U. 2001. Neogenesis of cerebellar Purkinje neurons from gene-marked bone marrow cells in vivo. J Cell Biol 155:733–8.

Recio JS, Álvarez-Dolado M, Díaz D, Baltanás FC, Piquer-Gil M, Alonso JR, and others. 2011. Bone marrow contributes simultaneously to different neural types in the central nervous system through different mechanisms of plasticity. Cell Transplant 20:1179–92.

Reitz M, Demestre M, Sedlacik J, Meissner H, Fiehler J, Kim SU, and others. 2012. Intranasal delivery of neural stem/progenitor cells: a noninvasive passage to target intracerebral glioma. Stem Cells Transl Med 1:866–73.

Rodríguez M, Álvarez-Erviti L, Blesa FJ, Rodríguez-Oroz MC, Arina A, Melero I, and others. 2007. Bone-marrow-derived cell differentiation into microglia: a study in a progressive mouse model of Parkinson's disease. Neurobiol Dis 28:316–25.

Romanko MJ, Rothstein RP, Levison SW. 2004. Neural stem cells in the subventricular zone are resilient to hypoxia/ischemia whereas progenitors are vulnerable. J Cereb Blood Flow Metab 24:814–25.

Sadat K, Ather S, Aljaroudi W, Heo J, Iskandrian AE, Hage FG. 2014. The effect of bone marrow mononuclear stem cell therapy on left ventricular function and myocardial perfusion. J Nucl Cardiol 21:351–67.

Scheulen ME, Hilger RA, Oberhoff C, Casper J, Freund M, Josten KM, and others. 2000. Clinical phase I dose escala-

tion and pharmacokinetic study of high-dose chemotherapy with treosulfan and autologous peripheral blood stem cell transplantation in patients with advanced malignancies. Clin Cancer Res 6:4209–16.

Sharma A, Gokulchandran N, Chopra G, Kulkarni P, Lohia M, Badhe P, and others. 2012. Administration of autologous bone marrow-derived mononuclear cells in children with incurable neurological disorders and injury is safe and improves their quality of life. Cell Transplant 21(Suppl 1): S79–S90.

Shinohara C, Gobbel GT, Lamborn KR, Tada E, Fike JR. 1997. Apoptosis in the subependyma of young adult rats after single and fractionated doses of X-rays. Cancer Res 57:2694–702.

Siqueira RC, Messias A, Voltarelli JC, Messias K, Arcieri RS, Jorge R. 2013. Resolution of macular oedema associated with retinitis pigmentosa after intravitreal use of autologous BM-derived hematopoietic stem cell transplantation. Bone Marrow Transplant 48:612–3.

Sjoo F, Hassan Z, Abedi-Valugerdi M, Griskevicius L, Nilsson C, Remberger M, and others. 2006. Myeloablative and immunosuppressive properties of treosulfan in mice. Exp Hematol 34:115–21.

Strother DR, Pollack IF, Gisher PG, Hunter JV, Woo SY, Pomeroy SL, and others. 2002. Tumors of the central nervous system. In: Pizzo PA, Poplack DG, editors. Principles and practice of pediatric oncology. Philadelphia: Lippincott Williams & Wilkins. p. 751–824.

Tada E, Yang C, Gobbel GT, Lamborn KR, Fike JR. 1999. Long-term impairment of subependymal repopulation following damage by ionizing irradiation. Exp Neurol 160:66–77.

Thomas ED. 1983. Bone marrow transplantation in leukemia. Haematol Blood Transfus 28:11–5.

Thompson M, Wall DM, Hicks RJ, Prince HM. 2005. In vivo tracking for cell therapies. Q J Nucl Med Mol Imaging 49:339–48.

Tögel F, Westenfelder C. 2007. Adult bone marrow-derived stem cells for organ regeneration and repair. Dev Dyn 236:3321–31.

Travlos GS. 2006. Normal structure, function, and histology of the bone marrow. Toxicol Pathol 34:548–65.

Vallieres L, Sawchenko PE. 2003. Bone marrow-derived cells that populate the adult mouse brain preserve their hematopoietic identity. J Neurosci 23:5197–207.

van Velthoven CT, Kavelaars A, van Bel F, Heijnen CJ. 2010. Nasal administration of stem cells: a promising novel route to treat neonatal ischemic brain damage. Pediatr Res 68:419–22.

Venkataramana NK, Kumar SK, Balaraju S, Radhakrishnan RC, Bansal A, Dixit A, and others. 2010. Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. Transl Res 155:62–70.

Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. 2002. Little evidence for developmental plasticity of adult hematopoietic stem cells. Science 297:2256–9.

Wehner T, Bontert M, Eyupoglu I, Prass K, Prinz M, Klett FF, and others. 2003. Bone marrow-derived cells expressing green fluorescent protein under the control of the glial fibrillary acidic protein promoter do not differentiate into astrocytes in vitro and in vivo. J Neurosci 23:5004–11.

Wei N, Yu SP, Gu X, Taylor TM, Song D, Liu XF, and others. 2013. Delayed intranasal delivery of hypoxic-preconditioned bone marrow mesenchymal stem cells enhanced cell homing and therapeutic benefits after ischemic stroke in mice. Cell Transplant 22:977–91.

Weimann JM, Charlton CA, Brazelton TR, Hackman RC, Blau HM. 2003a. Contribution of transplanted bone marrow cells to Purkinje neurons in human adult brains. Proc Natl Acad Sci U S A 100:2088–93.

Weimann JM, Johansson CB, Trejo A, Blau HM. 2003b. Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. Nat Cell Biol 5:959–66.

Wiersema A, Dijk F, Dontje B, van der Want JJ, de Haan G. 2007. Cerebellar heterokaryon formation increases with age and after irradiation. Stem Cell Res 1:150–4.

Wright DE, Wagers AJ, Gulati AP, Johnson FL, Weissman IL. 2001. Physiological migration of hematopoietic stem and progenitor cells. Science 294:1933–6.

Yamanaka S, Blau HM. 2010. Nuclear reprogramming to a pluripotent state by three approaches. Nature 465:704–12.

Yuan H, Gaber MW, Boyd K, Wilson CM, Kiani MF, Merchant TE. 2006. Effects of fractionated radiation on the brain vasculature in a murine model: blood-brain barrier permeability, astrocyte proliferation, and ultrastructural changes. Int J Radiat Oncol Biol Phys 66:860–6.