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Cell-based therapies for amyotrophic lateral sclerosis/motor neuron disease (Review)

Abdul Wahid SF, Law ZK, Ismail NA, Azman Ali R, Lai NM

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TABLE OF CONTENTS

[Intervention Review]

Cell-based therapies for amyotrophic lateral sclerosis/motor neuron disease

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ABSTRACT

Background

Amyotrophic lateral sclerosis (ALS), which is also known as motor neuron disease (MND) is a fatal disease associated with rapidly progressive disability, for which no definitive treatment as yet exists. Current treatment regimens largely focus on relieving symptoms to improve the quality of life of those affected. Based on data from preclinical studies, cell-based therapy is a promising treatment for ALS/MND.

Objectives

To assess the effects of cell-based therapy for people with ALS/MND, compared with placebo or no additional treatment.

Search methods

On 21 June 2016, we searched the Cochrane Neuromuscular Specialised Register, CENTRAL, MEDLINE, and Embase. We also searched two clinical trials' registries for ongoing or unpublished studies.

Selection criteria

We planned to include randomised controlled trials (RCTs), quasi-RCTs and cluster RCTs that assigned people with ALS/MND to receive cell-based therapy versus a placebo or no additional treatment. Co-interventions were allowable, provided that they were given to each group equally.

Data collection and analysis

We followed standard Cochrane methodology.

Main results

No studies were eligible for inclusion in the review. We identified four ongoing trials.

Authors' conclusions

Currently, there is a lack of high-quality evidence to guide practice on the use of cell-based therapy to treat ALS/MND.

We need large, prospective RCTs to establish the efficacy of cellular therapy and to determine patient-, disease- and cell treatment-related factors that may influence the outcome of cell-based therapy. The major goals of future research should be to determine the appropriate cell source, phenotype, dose, and route of delivery, as these will be key elements in designing an optimal cell-based therapy programme for people with ALS/MND. Future research should also explore novel treatment strategies, including combinations of cellular therapy and standard or novel neuroprotective agents, to find the best possible approach to prevent or reverse the neurological deficit in ALS/MND, and to prolong survival in this debilitating and fatal condition.

PLAIN LANGUAGE SUMMARY

Cell-based therapies for amyotrophic lateral sclerosis/motor neuron disease (ALS/MND)

Review question

How effective and safe is cell-based therapy in people with ALS/MND, when we compare it with an inactive treatment or no treatment?

Background

ALS/MND is a condition in which nerves in the brain and spinal cord that control movement (motor neurons) stop working. A person with ALS/MND has difficulty moving, swallowing, chewing and speaking, which become worse over time. Half of people with ALS/MND die within three years of their first symptoms. Weakness of muscles used in breathing often leads to death. The condition currently has no cure. Current treatment regimens largely focus on relieving symptoms to improve the quality of life of those affected. Cell-based therapy can be defined as injection of cellular material into a person to treat disease. Based on early studies, cell-based therapy is a promising new treatment. Various types of cell-based therapies can be used in ALS/MND, including stem cell therapy. Stem cell therapy aims to provide new motor neurons, which may help stop or slow down disease progression in people with ALS.

Study characteristics

We searched multiple databases for clinical trials.

Key results and quality of the evidence

We did not find any completed randomised controlled trials that assessed the effects of cell-based therapy. Four trials are in progress. As early studies are promising, we urgently need large, well-designed clinical trials to establish whether cell-based therapies have clinical benefit in ALS/MND. Major goals of future research are to identify the right type and amount of cells to use, and how best to administer them.

The evidence is up to date as of 21 June 2016.

BACKGROUND

Description of the condition

Motor neuron disease (MND) is a rare neurodegenerative disorder with an annual incidence of approximately 2 per 100,000 population. MND affects men and women of all ages, with a peak incidence at 50 to 70 years of age (Logroscino 2005; Logroscino 2008). The cause of MND is unknown, but up to 10% of cases are familial (Murray 2004). The clinical features of MND are attributable to the degeneration of neurons and corticospinal tracts from the primary motor cortex in the brain to the anterior horn cells in the spinal cord and brainstem nuclei (Rabin 1999). Four major categories of MND are recognised, namely amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS), progressive

muscular atrophy (PMA), and progressive bulbar palsy (PBP). When the person presents with both upper and lower motor neuron signs, the disease is known as ALS, which is the most common form of MND. The terms PLS and PMA are applied when the initial presentation reflects only upper motor neuron involvement or only lower motor neuron involvement, respectively. PBP presents with weakness of bulbar muscles. Common clinical features of MND include wasting and weakness of the muscles for mastication, speech articulation and swallowing, intrinsic muscles of the hands and muscles of lower limbs. Respiratory failure due to respiratory muscle weakness is a late feature, leading to death (Caroscio 1987). Rarely, ALS/MND presents with acute respiratory failure (Chen 1996). The disease is virtually always fatal. Approximately half of people with ALS/MND die within three years from onset of symptoms, although 10% of people with ALS/MND live longer than 10 years (del Aguila 2003; Turner 2003).

The exact mechanism leading to selective cell death of motor neurons is not well understood and is likely to be multifactorial, involving genetic and environmental factors. Several genes have been identified as the cause of familial ALS, including mutations in Cu^{2+}/Zn^{2+} superoxide dismutase 1 (SOD1), TAR DNA-binding protein 43 (TARDBP), fused in sarcoma (FUS), and c9orf72 (Renton 2014). However, the genetic defect in sporadic ALS is still unknown. The neurodegenerative process of MND may involve a complex interplay between genetic factors, oxidative stress, glutamatergic excitotoxicity, protein aggregation, mitochondrial dysfunction, and impairment of axonal transportation. The surrounding glial cells have also been implicated in pathogenesis via the release of inflammatory mediators, impaired neuronal metabolic support, and dysfunctional signalling pathways (Lunn 2014; Shaw 2005). All these processes eventually lead to apoptosis of motor neurons.

To date, there is no curative treatment for MND. Current treatment regimens focus on relieving symptoms to improve the quality of life of those affected. Riluzole, an antiglutamate agent, is the only available pharmacological treatment for ALS. It has a small beneficial effect on bulbar function, limb function, and survival, but no effect on muscle strength (Bensimon 1994; Goodall 2006; Miller 2012). Many other pharmacological agents have been tried, but without clear benefit. In addition non-pharmacological treatment, such as non-invasive ventilation, prolongs median survival and improves quality of life in people with ALS/MND (Bourke 2006; Radunovic 2013).

Description of the intervention

Multipotential stem cells may provide an attractive therapeutic option because of their ability to migrate into damaged neural tissues and promote regeneration of neurons (neurogenesis). These multipotential stem cells produce neurotrophic (growth-stimulating) factors, thus provoking the transdifferentiation of stem cells into neurons (Karussis 2010).

Cell-based therapy can be defined as injection of cellular material into someone for therapeutic purposes. Various type of cells can be used including stem cells that are used to treat degenerative diseases (regenerative medicine), blood cancers, and bone marrow diseases (bone marrow transplantation). To date, there have been numerous clinical trials of the treatment of ALS/MND with cell-based therapy utilising cells isolated mostly from autologous (the person's own) bone marrow and peripheral blood, thus minimising the risk of rejection. The types of cells used for implantation have been bone marrow mononuclear cells (BM-MNCs; Blanquer 2012; Deda 2009; Prabhakar 2012), bone marrow-derived mesenchymal stem cells (BM-MSCs; Baek 2012; Blanquer 2012; Karussis 2010; Martinez 2012; Mazzini 2003; Mazzini 2006; Mazzini 2012), granulocyte-colony stimulating factor (G-CSF)-mobilised-peripheral blood mononuclear cells (M-PBMNCs; Cashman 2008; Chio 2011; Nefussy 2010), olfactory ensheathing stem cells (OESC; Chen 2007; Chew 2007; Giordana 2010; Huang 2008; Piepers 2010), and neural stem cells (NSCs; Feldman 2014).

BM-MNCs are usually separated by a density gradient method from bone marrow aspirate obtained from the individual's hip bone. Mesenchymal stem cells (MSCs) can be easily isolated from bone marrow, placenta, muscle, and fat. The cells are subsequently cultured for three to five weeks to provide large numbers for therapeutic application. These cells can be expanded in vitro with no risk of malignant transformation (Bernardo 2007). The process of obtaining M-PBMNCs involves administration of G-CSF to increase the number of M-PBMNCs in the circulation, followed by their removal using a blood cell separation machine (apheresis). OESCs are extracted from human fetal olfactory bulb tissue and cultured for two to three weeks. NSCs used in clinical studies are cultured human NSCs derived from a single source human fetal spinal cord tissue of approximately eight gestational weeks and expanded serially by epigenetic means only (Feldman 2014).

Implantation of cells has been performed via several routes. The common methods include intrathecal (into the subarachnoid space via the spinal canal), intracortical (into the cerebral cortex), and direct transplantation of autologous MSCs into surgically-exposed spinal cord under general anaesthesia. Studies have shown that direct transplantation of autologous cells into the spinal cord is well tolerated and feasible in people with ALS (Feldman 2014; Mazzini 2012).

A number of clinical trials have provided important insights into the safety and feasibility of stem cell mobilisation and transplantation in people with ALS/MND. Uncertainties remain, however, regarding its ability to achieve functional improvement and its long-term safety profile; in particular, whether this mode of therapy is associated with acceleration of disease progression (Lunn 2014).

How the intervention might work

There are two possible mechanisms by which stem cell therapy may help in the treatment of ALS/MND. Firstly, by using progenitor cells that have been generated ex vivo to regenerate dying neuronal cells. Experimental observations showed that transplanted stem cells and mononuclear cells have the capacity to stimulate the regenerative processes of motor neurons (Mazzini 2003). In animal models of ALS, stem cell transplantation can significantly slow the progression of the disease and prolong survival (Mazzini 2003). Increasing numbers of preclinical studies have shown that transplanted stem cells are capable of migrating to regions of experimentally-induced nerve injury, where they are able to proliferate and differentiate into neurons and glial cells (Jiang 2002; Liu 2000; McDonald 1999; Terada 2002; Woodbury 2000). The types of stem cell that have been tested in preclinical models include BM-MSCs, MSCs, cord blood stem cells, embryonic stem cells, neural stem and progenitor cells, human glial restricted progenitors, and induced pluripotent stem cells (IPCs).

Secondly, stem cells promote the survival of existing neurons. MSCs are very attractive candidates for cell therapy in MND because of their great plasticity (Chen 2008), and immunomodulatory properties (Mazzini 2012). MSCs can induce a neuroprotective microenvironment via anti-inflammatory and immunosuppressive effects on astrocytes and microglial cells (Uccelli 2008). MSCs release soluble molecules such as cytokines and chemokines, and express immune-relevant receptors such as chemokine receptors and cell adhesion molecules that ameliorate inflammation and stimulate the survival of neuronal cells (Uccelli 2008). Preclinical data have shown that MSCs are capable of transdifferentiation into neurons and glial cells both in vitro and in vivo (Black 2001; Kim 2002; Sanchez-Ramos 2000). In addition, neural stem cells have the ability to generate immunomodulatory cells, growth-factor-releasing cells and functional support cells to modify motor neuron survival and activity (Gowing 2011).

Most studies on the pathogenesis of ALS thus far have been in animals. There are many limitations when extrapolating the findings observed in animal models into humans. Firstly, there are interspecies differences in neuronal physiology and specific gene splicing patterns (Hardingham 2010). Secondly, there is an overemphasis on models based on rat superoxide dismutase 1 (SOD1), when most cases of human sporadic ALS may not have a SOD1 defect. In this respect, stem cells could be used to model disease, allowing us to further explore the pathophysiological process of ALS.

Why it is important to do this review

The lack of effective pharmacologic treatment for ALS/MND and compelling preclinical data have provided a rationale for the therapeutic application of stem cells for this devastating incurable disease. Early clinical trials have suggested that stem cells could have the potential to replace and repair damaged motor neurons in people with ALS/MND (Martinez 2012; Mazzini 2003; Mazzini 2015). Moreover, the procedures of expansion and transplantation of these cells into people with ALS/MND are well tolerated and feasible. However, most of the clinical trials involved small numbers of participants, which can produce false-positive results, or overestimate the magnitude of an association; consequently, the results have been inconsistent. Additionally, small trials may fail to detect rare adverse events. Combining available data in a systematic review may increase the likelihood of detecting a true effect of the intervention, thus allowing meaningful conclusions to be drawn. It is also important to know whether cells derived from different sources have different impacts on clinical outcomes among people with ALS/MND. For example, stem cells obtained from different sources may possess different biological properties (plasticity, self-renewal, differentiation, homing, migration, and secretion of trophic factors) and different immunological properties (modulating immune response). These differences may be attributed to the inherent biological properties of the stem cells or changes that occur during enrichment and processing. Moreover, questions regarding the optimal treatment regimen, including the cell dose, phenotype, preparation, and delivery system, remain to be answered (Abdul 2013).

This systematic review sets out to determine the efficacy, feasibility and safety of cell-based therapy in people with ALS/MND. The findings of this review may facilitate design of the optimal cell-based therapy programme for people with ALS/MND as well as identify critical areas for improvement, and recommendations for future clinical trials.

OBJECTIVES

To assess the effects of cell-based therapy in people with ALS/MND compared with a placebo or no additional treatment.

METHODS

Criteria for considering studies for this review

Types of studies

We planned to include randomised controlled trials (RCTs), quasi-RCTs and cluster RCTs. Quasi-random methods of assignment to interventions are systematic methods that are not truly random, such as allocation using alternation, date of birth, day of visit, or medical record number.

Types of participants

We planned to include people of any age with a diagnosis of definite or probable ALS/MND according to accepted criteria, such as the revised El Escorial World Federation of Neurology criteria (Brooks 2000).

Types of interventions

Mononuclear cells or stem cells compared with i) a placebo or ii) no additional treatment. We would have permitted the use of co-interventions including standard treatment such as riluzole and symptomatic treatment, provided that they were administered to each group equally.

Types of outcome measures

Primary outcomes

1. Change in functional rating scale, such as the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) at 6 months (Cedarbaum 1999).

Secondary outcomes

1. Change in functional rating scale, such as the ALSFRS-R at 12 months (Cedarbaum 1999).
2. Change in manual muscle testing of the upper and lower limbs (Medical Research Council (MRC) grade) at 6 and 12 months.
3. Change in upright forced vital capacity (FVC) at 6 and 12 months.
4. Change in compound muscle action potential (CMAP), neurophysiological index (NI), combined motor index (CMI), motor unit number estimation (MUNE) and motor unit number index (MUNIX) at 6 and 12 months (Escorcio-Bezerra 2016; Gawel 2016; Stein 2016).
5. Change in mood state and quality of life using the Profile of Mood State (POMS) and quality of life scale questionnaires (such as ALS Assessment Questionnaires, ALSAQ-40 or ALSQ5, Short-Form 36 (SF-36) Health Survey and EQ-5D) at 6 and 12 months (Jenkinson 2000; Jenkinson 2007; Rabin 2001; Ware 1992).
6. Structural changes in serial magnetic resonance imaging (MRI) such as T2-weighted and Fluid-Attenuated Inversion Recovery (FLAIR) hyperintense signals in corticospinal tracts, precentral and frontal cortex at 6 and 12 months.
7. Overall survival at 6 and 12 months.
8. Adverse events include an inflammatory reaction at the cell injection site, and cardiovascular and thromboembolic complications. We would have reported the rate of adverse events and the rate of withdrawal from the study.

Search methods for identification of studies

Electronic searches

On 21 June 2016, the Cochrane Neuromuscular Information Specialist searched the Cochrane Neuromuscular Specialised Register, CENTRAL (21 June 2016 in the Cochrane Register of Studies Online (CRSO)), MEDLINE (January 1966 to June 2016) and Embase (January 1980 to June 2016), without applying any language restrictions. The detailed search strategies are in the appendices: Cochrane Neuromuscular Specialised Register Appendix 1; CENTRAL Appendix 2; MEDLINE Appendix 3; and Embase Appendix 4. We included studies reported as full-text publications as well as those published as abstracts and proceedings.

We also conducted a search of the US National Institutes of Health Clinical Trials Registry (www.clinicaltrials.gov), and the World Health Organization International Clinical Trials Registry Platform (ICTRP; apps.who.int/trialsearch/) to identify other ongoing and unpublished studies. In addition, we searched the National Institute for Health Research Database of Abstracts and Reviews of Effects (DARE) and Health Technology Assessments (HTA) database to identify reviews and assessments for inclusion in the 'Discussion' section. We searched National Health Service Economic Evaluation Database (NHS EED) for any available cost information for the 'Discussion' section.

Searching other resources

We searched for additional references in reference lists of all primary studies and review articles. We contacted the authors of RCTs and other experts in the field to obtain any additional published or unpublished studies. We searched relevant manufacturers' websites for trial information to identify further relevant studies. In addition, we handsearched journals for relevant articles: Cytotherapy (January 1999 to 21 June 2016), Cell Transplantation (Issue 1 2001 to issue 6 2016), Cell Stem Cell (Issue 1, 2007 to Issue 6, 2016) and Stem Cells (Issue 1, 1993 to Issue 6, 2016).

Data collection and analysis

Selection of studies

Two review authors (SFAW and ZKL) independently screened titles and abstracts of all studies identified from the first round of searching. We coded potentially relevant studies or studies that required further assessments as 'retrieve' based on the relevance of the population, intervention and outcomes to our review question. We coded studies clearly not relevant as 'do not retrieve'. Two review authors (SFAW and ZKL) inspected the full-text versions

of the studies coded as 'retrieve' to further identify trials to be included in our meta-analysis, based on the relevance of the population, intervention, comparison, and the study design. Among studies retrieved but excluded, we recorded reasons for exclusion. We resolved any disagreement through discussion and did not require consultation with a third person. We identified and excluded duplicates, and collated multiple reports of the same study, making each study the unit of interest in the review rather than each report. We recorded the selection process in sufficient detail to complete a PRISMA flow diagram and Characteristics of excluded studies table.

We did not identify any included studies for quantitative analysis. We provide a qualitative narration of the excluded studies in the Discussion.

Data extraction and management

If we had identified any eligible studies, we would have applied the methods described in our protocol (Abdul Wahid 2015), and detailed in Appendix 6.

RESULTS

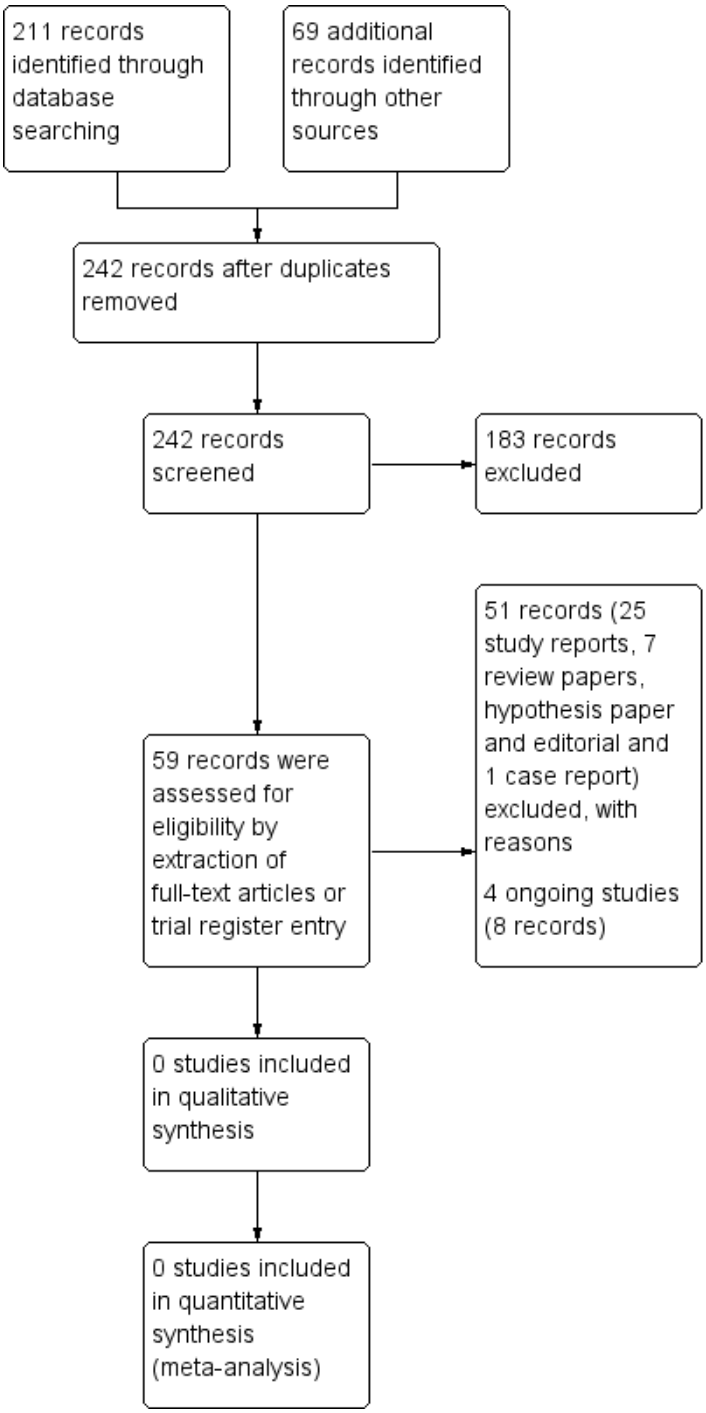
Description of studies

We identified no studies eligible for inclusion.

Results of the search

Our search yielded 280 records, including 211 from database searches and 69 from other sources. After deduplication, 242 records remained. We shortlisted 59 records for closer inspection, and excluded 51 records with reasons (see Characteristics of excluded studies). The remaining eight records described four ongoing trials (NCT02290886; NCT01254539; NCT02286011; NCT02017912) (see Characteristics of ongoing studies). So, from our current searches, we found no eligible study for inclusion. Figure 1 shows a summary of the results of the search.

Figure 1. Study flow diagram.



Included studies

None.

Excluded studies

We assessed and excluded 51 full-text articles describing 25 clinical trials (680 participants). These were as follows.

- Nineteen single-arm, non-randomised trials. These trials investigated the safety and efficacy of BM-MSC in 79 participants (Czaplinski 2015; Karussis 2010; Mazzini 2003; Mazzini 2012; Oh 2015), BM-MNC in 26 participants (Blanquer 2012; Boonyapisit 2009; Prabhakar 2012), bone marrow-derived hematopoietic stem cells in 13 participants (Deda 2009), granulocyte-colony stimulating factor (G-CSF)-mobilised-peripheral blood mononuclear cells (M-PBMNCs) in 8 participants (Cashman 2008), neural stem cells in 19 participants (Feldman 2014; Moviglia 2012), fetal human neural stem cells in 6 participants (Mazzini 2015), umbilical cord blood mesenchymal stem cells (CB-MSCs) in 100 participants (Miao 2015), mesenchymal stem cells secreting neurotrophic factors (MSC-NTF) in 38 participants (Karussis 2013; Petrou 2015), adipose tissue MSC in 12 participants (Staff 2014), CD133+ stem cells in 67 participants (Martinez 2012), and olfactory ensheathing stem cells (OESC) in 507 participants (Chen 2007).
- Three non-randomised controlled trials. Huang 2008 examined the safety and efficacy of OESC transplantation in 35 participants, Martinez 2009 evaluated CD133+ stem cells enriched from autologous peripheral blood in 20 participants. and Sharma 2015 investigated autologous BM-MNC in 57 participants.
- Three trials that examined clinical outcomes of G-CSF injection (Chio 2011; Grassinger 2014; Nefussy 2010).
- One case report (Baek 2012).
- Seven other review, hypothesis or editorial publications (Badayan 2008; Baig 2014; Bedlack 2011; Boulis 2011; Goyal 2014; Kim 2013; Thomsen 2014).

(See Characteristics of excluded studies)

Risk of bias in included studies

No studies were eligible for inclusion.

Effects of interventions

No studies were eligible for inclusion.

DISCUSSION

Summary of main results

We identified 25 published clinical trials (involving 680 participants) that assessed the safety, feasibility, and efficacy of cell-based therapy in amyotrophic lateral sclerosis (ALS). However, we found no randomised controlled trials (RCTs) or quasi-RCTs eligible for inclusion in this review. We therefore summarised findings of non-randomised studies.

Evidence from non-randomised studies

Relevant non-RCTs included 875 participants from 19 single-arm phase I to II trials and 112 participants from three controlled trials. They aimed mainly to assess the safety and feasibility of surgical procedures involved in the implantation of cells into the brain and spinal cord of people with ALS and to determine whether this mode of therapy is associated with acceleration of disease progression. These trials used various cell delivery methods: the most common route of cell administration was intraspinal (Blanquer 2012; Boonyapisit 2009; Deda 2009; Feldman 2014; Mazzini 2003; Mazzini 2012; Mazzini 2015), followed by intrathecal via lumbar puncture or Ommaya reservoir (Czaplinski 2015; Karussis 2010; Miao 2015; Oh 2015; Prabhakar 2012; Sharma 2015; Staff 2014). Other routes of cell administration included intracerebral injection (Chen 2007; Huang 2008; Martinez 2009; Martinez 2012), and combined intrathecal and intramuscular injection (Karussis 2013; Petrou 2015). In general, these trials reported only minor adverse events, including spinal headache, pain at injection and aspiration sites, nausea, vomiting, and fatigue. Notably, these studies found no long-term surgical complications and the participants did not show acceleration in disease progression related to cell implantation. Despite the results, which appeared promising, there are great uncertainties on the findings due to the inherent limitations of these studies. Non-randomised studies can give rise to imbalances in factors that may influence the treatment outcome between groups being compared, thus may introduce bias and uncertainty to the estimates of treatment effect.

Studies have evaluated a variety of cell-based products designed to either replace the lost motor neurons or improve the metabolic supply of the affected neurons, thus delaying their death. Mesenchymal stem cells (MSCs), obtained mainly from autologous bone marrow (BM), is the most frequently-used cell type in clinical trials because it is readily available in large numbers, it releases growth factors, and it is non-immunogenic. In people with ALS, no serious side-effects and no detrimental effects on neurological function occurred following intraspinal transplantation of variable

doses of MSCs (7 to 152 × 10^6 cells). Some trials reported improvement in motor function in people with ALS (Karussis 2010; Oh 2015; Petrou 2015), and some reported no clinical benefit (Czaplinski 2015). MSCs have shown remarkable results in preclinical studies. However, efficacy data in humans are limited and phase II clinical trials are currently in progress.

Conflicting findings exist regarding the effect of olfactory ensheathing stem cell (OESC) transplantation in people with ALS/MND. Two non-randomised clinical studies conducted in the People's Republic of China reported outcomes in favour of intracranial OESC transplantation. Huang 2008 reported a significantly greater reduction in functional deterioration three to four months post OESC transplantation (n = 15) than in an untreated control group (n = 20); and a single-arm trial (N = 42) reported improvement in neurological and lung function after repeated cell administration (Chen 2007). However, other reports did not support these clinical observations (Chew 2007; Giordana 2010; Piepers 2010). One woman with ALS received intracranial injection of adult olfactory ensheathing glia (OEG) transplantation and her disease progressed at a more rapid rate after the procedure and she suffered disabling side-effects (Chew 2007). Piepers 2010 performed a prospective study of seven people who underwent OEC treatment in China. The reported improvement in three participants was temporary and was not detectable at six months after OEC treatment. In addition, two had severe side-effects and died 12 months after transplantation. Giordana 2010 reported postmortem findings on two participants with ALS who received intracranial injection of fetal olfactory ensheathing cells (OECs). The OEC transplantation did not modify the neuropathology of ALS and there was absence of axonal regeneration, neuronal differentiation and myelination.

The safety of cell-based therapies was the primary question in the majority of single-armed clinical trials; however, these trials provided important insight into the therapeutic potential of cell-based therapy in ALS/MND. Three trials involving approximately 35 participants with ALS reported that cell-based therapy slowed the rate of decline in Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) score (Feldman 2014; Karussis 2013; Oh 2015). Martinez 2009 showed a significant increment in ALSFRS-R scores at 1, 2, 6, and 12 months' follow-up compared with baseline values in the CD133+ stem cell treatment group (10 participants) and a significant decrease in the mean score at 6 and 12 months in the control group (10 participants).

Moreover, these trials also attempted to address the impact of cell treatment on survival of people with ALS/MND. Apart from riluzole and non-invasive ventilation, current management strategies showed very limited effect on survival in ALS/MND (Miller 2012; Radunovic 2013). Two non-RCTs have addressed the effect of cell treatment on survival in people with ALS (Martinez 2009; Sharma 2015). Martinez 2009 showed that median survival in the cell-based therapy group was significantly longer than in the control group (66 months versus 19 months, P = 0.0111, N = 10). Sharma 2015 was a retrospective controlled cohort study in which participants received standard rehabilitation therapy and riluzole, which showed an increase in survival of 30.38 months in a group treated with BM-MNCs (N = 37) compared to a control group who did not receive BM-MNCs (N = 20) (mean survival duration from the onset of disease was 87.76 ± 10.45 months in the BM-MNC group versus 57.38 ± 5.31 months in the control group). The survival of the group treated with BM-MNC was longer compared to previously reported survival of people treated with riluzole. A subgroup analysis of the intervention arm revealed that younger age at disease onset (< 50 years), limb onset (compared to bulbar onset) and concurrent lithium therapy were associated with longer survival. The impact of cell-based therapy on survival needs to be determined in future RCTs.

Currently, the data regarding the neuroprotective property of different cell-based products are limited. Furthermore the key elements, including cell source, phenotype, dose, and route of implantation, that will be critical in designing optimal cell-based therapy for people with ALS/MND remain unclear. Four ongoing RCTs are addressing some of these key issues related to cell treatment protocol in participants with ALS. These trials are expected to be completed between 2016 and 2018. NCT02290886 is a phase I/II randomised, placebo-controlled, triple-blind trial to evaluate the safety and efficacy of intravenous autologous adipose tissue-derived MSCs in three different doses (one million, two million, and four million). NCT02017912 is a phase II, randomised, double-blind, placebo-controlled multicentre trial to evaluate the safety and efficacy of implantation of autologous MSCs secreting neurotrophic factors (MSC-NTF) in participants with ALS. The trial also intends to compare the efficacy of intramuscular injection versus intrathecal injection of MSC-NTF. NCT01254539 is a double-blind RCT comparing intrathecal and intraspinal implantation of autologous bone marrow-derived stem cells versus intrathecal placebo. Finally, NCT02286011 is a double-blind RCT comparing intramuscular infusion of BM-MNCs with placebo in 20 participants with ALS.

Potential biases in the review process

We performed comprehensive searches in the Cochrane Neuromuscular Specialised Register, CENTRAL, MEDLINE, and Embase. Research conducted to answer the review question is still in its early stages, with several phase I and phase II studies identified, but no RCTs. Despite our comprehensive searches, it is possible that we missed some relevant articles and conference presentations not listed in the databases above or not captured in the handsearching process.

Agreements and disagreements with other studies or reviews

The findings of the present review appear in line with two preliminary studies that evaluated safety and feasibility of stem cell mobilisation and cell transplantation procedures into the brain, spinal cord and thecal sac of people with ALS/MND (Goutman 2015; Lunn 2014). These single-arm, small clinical phase I/II trials showed that, in general, direct cell implantation into the cerebral cortex, spinal cord and thecal sac of people with ALS/MND appears feasible with no association with acceleration in disease progression, although there are great uncertainties on the findings due to the non-randomised and preliminary nature of the trials.

These studies also demonstrated that stem cells can be mobilised successfully from people with ALS/MND without significant adverse effects related to G-SCF administration and aphaeresis (a procedure used to harvest peripheral blood stem cells). Cell administration via intracerebral, intraspinal, intrathecal, intramuscular, and intravenous routes are feasible and tolerable. Importantly, the trials found no immediate and long-term surgical complications related to cell implantation procedures; and participants did not show an acceleration in disease progression.

As most human studies to date focus on the safety of various types of cell-based products and the feasibility of the surgical implantation technique, these issues have been the focus of previous reviews. Despite the remarkable safety profile of cell-based therapy, data on efficacy in humans are still very limited. Previous reviews supported the use of cell-based therapy as a means of delaying the disease course in ALS, mainly based on preclinical animal models (Goutman 2015; Thonhoff 2009). Single-arm and small clinical trials observed no clinical benefits. Limited data from non-RCTs involving a small number of people with ALS and a short-term follow-up period suggested that cell-based therapy slowed the rate of disease progression.

In agreement with recent reviews of phase I and II clinical trials published from 2007 to 2014 (Goutman 2015; Lunn 2014), we could not identify any randomised controlled trials of cell-based therapy in people with ALS/MND. To date, no published meta-analysis has compared the effects of cell-based therapy and conventional treatment in people with ALS/MND. The impact of cell-based therapy derived from different sources and phenotypes, administered in different doses and routes on clinical outcomes, has never been systematically described. The therapeutic potential of cell-based therapies in ALS/MND has not been fully evaluated, given the paucity of high-quality clinical trials.

Uncertainties remain as to whether this mode of therapy is capable of restoring muscle function and slowing disease progression in ALS/MND.

AUTHORS' CONCLUSIONS

Implications for practice

There is no published high-quality evidence to evaluate the efficacy and safety of cell-based therapy in ALS/MND.

Implications for research

To date, there is no conclusive evidence that cell-based therapy alters the natural course of ALS/MND and prolongs survival. There were significant shortcomings related to the design of the currently available published studies. We found significant variability between trials with regards to selection criteria, outcome measures, and types of cells and routes of cell implantation. Moreover, these trials were generally underpowered to show any clinical benefit. Nonetheless, preliminary data from these earlier trials with short-term follow-up suggest a trend towards stabilisation of the disease. Prospective RCTs with larger sample size and longer-term follow-up are urgently required to assess the clinical benefits of cell-based therapy including improvement in disease progression and quality of life and prolongation of survival in people with ALS/MND. Importantly, data from well-designed trials might determine patient-, disease- and cell treatment-related factors that could potentially influence the clinical outcomes of cell-based therapy.

Questions remain as to the optimal cell source, phenotype and dose, as well as transplantation route and protocol that would be key elements in designing an optimal cell-based therapy programme for people with ALS/MND; these should be the major goals of future research.

Combination of cellular therapy with standard therapy (riluzole) or novel neuroprotective agents should also be explored to strengthen the therapeutic efficacy and to find the best possible approach to prevent or reverse the neurological deficit and to prolong survival of this otherwise debilitating and fatal condition.

Studies to investigate the mechanisms of cellular neuroprotection induced by cell implantation would be vital in developing an effective cell-based targeted therapy in ALS/MND. Future clinical trials should consider the following factors.

1. Improved trial designs and participant selection criteria with relevant clinical outcomes.

2. Long-term follow-up period to establish long-term safety and durability of the clinical benefit of cell-based therapy.

3. Standardisation of cell products used and cell implantation protocols.

4. Detailed characterisation of cells used for implantation including viability and immunophenotype.

5. In vivo cell tracking using advanced imaging technologies to provide insight into the survival and migratory potential of the grafted cells.

6. Post-mortem pathological analysis of brain or spinal cord specimens from people with ALS, to detect evidence of

neuroprotection or axonal regeneration of the diseased motor neurons.

7. Using novel cellular sources and treatment approaches (such as induced pluripotent stem cells, cell lines expressing neutrotrophic growth factors and combining MSC and NSC transplantation).

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* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Badayan 2008	Not a RCT. Review of MSC trials in people with ALS
Baek 2012	Not a RCT. Case report using autologous MSCs from bone marrow in ALS
Baig 2014	Not a clinical study but a hypothesis paper concerning neurodegenerative disease: Alzheimer's disease, Parkinson's disease, Huntington's disease, ALS, and multiple sclerosis
Bedlack 2011	Editorial regarding the use of stem cells for people with ALS
Blanquer 2012	Not a RCT. Open, single-arm, phase I trial to evaluate the feasibility and safety of intraspinal infusion of autologous BM-MNC in people with ALS
Boonyapisit 2009	Not a RCT. A trial to determine if single bone marrow stem cell transfusion is safe and improves respiratory function in MND. Published in abstract
Boulis 2011	Not a RCT. A review article regarding use of stem cells as a therapy for ALS
Cashman 2008	Not a RCT. Pilot study of G-CSF-mobilized peripheral blood stem cells in ALS
Chen 2007	Not a RCT. Single-arm trial of olfactory ensheathing cell transplantation for ALS
Chio 2011	Not a RCT. Multicenter, open-label pilot study of G-CSF in people with ALS
Czaplinski 2015	Not a RCT. Single-arm study to evaluate the safety, tolerability and therapeutic effects of transplanting MSCs into people with ALS. Published in abstract
Deda 2009	Not a RCT. Treatment of ALS by autologous bone marrow-derived hematopoietic stem cell transplantation, phase II study (single-arm study)
Feldman 2014	Not a RCT. Phase I trial using intraspinal neural stem cell transplantation in ALS
Goyal 2014	Not a RCT. A review article of recently completed, ongoing, and planned trials using existing and novel drugs in ALS
Grassinger 2014	Not a RCT. Administration of recombinant human G-CSF in people with ALS (single-arm study)
Huang 2008	Not a RCT. Pilot study of olfactory ensheathing cell transplantation for ALS. Neither participants nor assessors were blinded
Karussis 2010	Not a RCT. Phase I/II open clinical trial on safety and immunological effects of MSC transplantation in multiple sclerosis and ALS

(*Continued*)

Karussis 2013	Not a RCT. A phase I/II clinical trial to evaluate the safety and tolerability of intramuscular and intrathecal treatment with autologous MSCs differentiated to secrete neurotrophic factors (MSC-NTF) in people with ALS
Kim 2013	Not a RCT
Martinez 2009	Not a RCT
Martinez 2012	Not a RCT. Single-arm trial
Mazzini 2003	Not a RCT. Single-arm trial of autologous MSC in people with ALS
Mazzini 2012	Not a RCT. Phase I clinical trial. MSC transplantation in ALS: a long-term safety study
Mazzini 2015	Not a RCT. Phase I clinical trial using fetal human neural stem cells (hNSCs) from natural in utero death administered into the anterior horns of the spinal cord to test for the safety of both cells and neurosurgical procedures in people with ALS
Miao 2015	Not a RCT. Clinical study investigating intrathecal administration of umbilical cord mesenchymal stem cells (UC-MSCs) by lumbar puncture, and effects in various neurological conditions
Moviglia 2012	Not a RCT. Single-arm trial of neural stem cells in ALS
Nefussy 2010	Non-randomised pilot study. Recombinant human G-CSF administration for treating ALS
Oh 2015	Not a RCT. Open-label, single-arm, phase I trial of repeated intrathecal autologous bone marrow-derived MSCs in ALS
Petrou 2015	Not a RCT. A study in amyotrophic lateral sclerosis (ALS) to evaluate the safety and efficacy of transplantation of autologous bone marrow-derived MSCs induced to secrete neurotrophic factors
Prabhakar 2012	Not a RCT. Single-arm, open-label trial
Sharma 2015	Retrospective controlled cohort study to compare the length of survival of participants who underwent transplantation of bone marrow mononuclear cells and a control group that did not receive cell transplantation
Staff 2014	Not a RCT. Dose-escalation safety trial on intrathecal delivery of autologous fat-derived MSCs in ALS. Published in an abstract
Thomsen 2014	Not a RCT. Review article

ALS: amyotrophic lateral sclerosis
BM-MNC: bone marrow mononuclear cells
G-CSF: granulocyte-colony stimulating factor
MND: motor neuron disease
MSC: mesenchymal stem cell
RCT: randomised controlled trial

Characteristics of ongoing studies *[ordered by study ID]*

NCT01254539

Trial name or title	Clinical trial on the use of autologous bone marrow stem cells in amyotrophic lateral sclerosis (extension CMN/ELA)
Methods	Randomised, double-blind study
Participants	**Inclusion criteria** • Diagnosis established using the World Federation of Neurology criteria • More than 6 and less than 36 months of evolution of the disease • Bulbar onset • Over 18 and under 70 years old • FVC ≥ 50% • Total time of oxygen saturation < 90% inferior to 5% of the sleeping time • Signed informed consent **Exclusion criteria** • Neurological or psychiatric disease • Need of parenteral or enteral nutrition through percutaneous endoscopic gastrostomy or nasogastric tube • Concomitant systemic disease • Treatment with corticosteroids, immunoglobulins or immunosuppressors in the last 12 months • Inclusion in other clinical trials • Inability to understand informed consent
Interventions	**Treatment arms** • Autologous bone marrow stem cells intraspinal transplantation at T3-T4 • Intrathecal infusion of autologous bone marrow stem cells • Intrathecal infusion of placebo (saline solution) Cell dose not mentioned.
Outcomes	**Primary outcome measures** 1. FVC **Secondary outcome measures** 1. Neurological variables: ALSFRS, MRC and Norris scales 2. Absence of adverse events 3. Neurophysiological variables: electromyography, polysomnography, evoked potentials 4. Neuroradiological variables: spinal magnetic resonance imaging (MRI) 5. Respiratory variables: maximal inspiratory pressure, maximal expiratory pressure (PEM), sniff nasal, oxymetry 6. Psychological variables: Health Questionnaire (EuroQol-5D), Profile of Mood States (POMS)
Starting date	October 2010 (duration: 62 months)
Contact information	Moraleda Jiménez JM, Hospital Universitario Virgen de la Arrixaca
Notes	

NCT02017912

Trial name or title	A phase II, randomised, double blind, placebo controlled multicentre study to evaluate safety and efficacy of transplantation of autologous mesenchymal stem cells secreting neurotrophic factors (MSC-NTF) in people with ALS
Methods	Randomised, double-blind, placebo-controlled, multicentre study
Participants	**Inclusion criteria** 1. Men and women aged 18 to 75 years old, inclusive 2. ALS diagnosed as possible, laboratory-supported probable, probable, or definite, as defined by revised El Escorial criteria 3. Disease onset, as defined by first reported occurrence of symptomatic weakness, spasticity, or bulbar symptoms, of 12 months and ≤ 24 months 4. Current disease symptoms must include limb weakness 5. ALSFRS-R ≥ 30 at the screening visit 6. Upright slow vital capacity measure ≥ 65% of predicted for gender, height, and age at the screening visit 7. Participants must be taking a stable dose of riluzole for at least 30 days prior to enrolment or not be on riluzole, and not have been on it for at least 30 days prior to enrolment (riluzole-naïve participants permitted in the study) 8. Capable of providing informed consent and willing and able to follow study procedures, including willingness to undergo lumbar puncture 9. Geographic accessibility to the study site and willingness and ability to comply with follow-up 10. Women of child-bearing potential must agree not to become pregnant for the duration of the study. Women must be willing to consistently use two forms of contraceptive therapy throughout the course of the trial, and undergo a pregnancy test 1 week before bone marrow aspiration. Men must be willing to consistently use 2 forms of contraceptive if their partners are of child-bearing age 11. Citizen or permanent resident of the United States **Exclusion criteria** 1. Prior stem cell therapy of any kind 2. Inability to lie flat for the duration of intrathecal cell transplantation or bone marrow biopsy, or inability to tolerate study procedures for any other reason 3. History of autoimmune disease (excluding thyroid disease), myelodysplastic or myeloproliferative disorder, leukemia or lymphoma, whole body irradiation, hip fracture, or severe scoliosis 4. Any unstable clinically significant medical condition other than ALS (e.g. myocardial infarction, angina pectoris, or congestive heart failure within 6 months of baseline), treatment with anticoagulants that, in the opinion of the investigator, would compromise the safety of participants 5. Any history of malignancy including any malignancy affecting the central nervous system and melanoma, within the previous 5 years, with the exception of localized skin cancers (with no evidence of metastasis, significant invasion, or re-occurrence within 3 years of baseline) 6. Serum AST or ALT values more than 3 times the upper normal limit 7. Serum creatinine value more than 2 times the upper normal limit 8. Positive test for HBV, HCV, HIV 9. Current use of immunosuppressant medication or use of such medication within 4 weeks of screening visit 10. Any history of acquired or inherited immune deficiency syndrome 11. Exposure to any other experimental agent (off-label use or investigational), or participation in a clinical trial within 30 days prior to screening visit 12. Use of non-invasive ventilation, diaphragm pacing system or invasive ventilation (tracheostomy)

NCT02017912 (*Continued*)

	13. Any history of either substance abuse within the past year or unstable psychiatric disease according to investigators' judgment 14. Placement or usage of feeding tube 15. Pregnant or currently breastfeeding
Interventions	**Treatment arms** • Autologous MSC-NTF cells • Placebo Cell dose not mentioned
Outcomes	**Primary outcome measure** 1. Number of participants with adverse events **Secondary outcome measures** 1. Change in ALSFRS slopes from the pre-transplantation period to the post-transplantation period between the treatment and placebo groups through 24 weeks post transplantation 2. Change in slow vital capacity slopes from the pre-transplantation period to the post-transplantation period between the treatment and placebo groups through 24 weeks post transplantation
Starting date	May 2014
Contact information	Cudkowicz M, Brown RH, Windebank AJ
Notes	

NCT02286011

Trial name or title	Intramuscular infusion of autologous bone marrow stem cells in people with amyotrophic lateral sclerosis (TCIM/ELA)
Methods	Prospective, randomised, double-blind study
Participants	**Inclusion criteria** • Diagnosis of definite or probable ALS according to the criteria established by the World Federation of Neurology • Reasonable assurance of adherence to protocol • Neurophysiological data confirming lower motor neuron lesions in the lumbar region • Assessment of motor deficits in dorsiflexion of both feet (4 or 5 points on the MRC scale; MRC scale grade muscle power as 0 = no contraction, 1 = flicker of contraction, 2 = active movement, with gravity eliminated, 3 = active movement against gravity, 4 = active movement against gravity and resistance, and 5 = normal power) • The participant must fulfil all inclusion criteria **Exclusion criteria** • Diabetes mellitus • Other diseases that may present with polyneuropathy • Previous history of stroke • Prior pathology of the peripheral nervous system affecting one or both lower limbs with or without clinically evident neurological sequelae • Pregnant or breastfeeding women

NCT02286011 (*Continued*)

	• Women physiologically capable of becoming pregnant, unless they are using reliable contraception • People with cardiac, renal, hepatic, systemic or immune conditions that could influence survival during the test • Positive serology for hepatitis B, hepatitis C or HIV • Clinical and anaesthesiological criteria contraindicating either sedation or extraction of bone marrow (an altered coagulation system or anticoagulated with inability to withdraw anticoagulation, haemodynamic instability, altered skin puncture site, etc.) • Included in other clinical trials in the last 6 months
Interventions	**Treatment arms** Intervention: intramuscular infusion of autologous BM-MNC (550 million cells (100 to 1200 million) diluted in 2 ml saline) in tibialis anterior muscle of one of the lower limbs Control: intramuscular infusion of placebo (2 ml saline) in the contralateral lower limb [sic]
Outcomes	**Primary outcome measures** 1. Rate of serious and non-serious adverse events related to cellular therapy in participants with ALS **Secondary outcome measures** 1. Estimated number of motor units (MUNE) 2. Compound muscle action potential (CMAP) 3. Fibre density 4. Muscle strength: MRC score 5. Maximum force developed in an isometric contraction of the tibialis anterior muscle 6. Maximum transversal area of the tibialis anterior muscle
Starting date	November 2014 (duration: 38 months)
Contact information	Natalia García Iniesta +34968381221 nagarini@yahoo.es
Notes	

NCT02290886

Trial name or title	Clinical trial phase I/II, randomised, controlled with placebo, triple blind to evaluate safety, and indications of efficiency of the intravenous administration of the therapy with 3 doses of MSC in participants with moderate to severe ALS
Methods	Clinical trial phase I/II, multicentre, randomised, placebo-controlled, triple-blind study
Participants	**Inclusion criteria** 1. Men and women over 18 years old 2. Good understanding of the protocol and able to grant informed consent 3. Definite or probable diagnosis of sporadic ALS in agreement with the criteria of El Escorial criteria, of the World Federation of Neurology 4. FVC of at least 50% of normal for sex, height and age 5. Disease onset (beginning of symptoms) > 6 months and < 36 months previously 6. Possibility of obtaining at least 50 g of adipose tissue 7. Treatment with riluzole for at least a month before inclusion **Exclusion criteria** 1. Any concomitant disease that under investigator's criteria could affect measures of the clinical variables

NCT02290886 (*Continued*)

	(hepatic, renal or cardiac insufficiency, diabetes mellitus, etc.) 2. Previous therapy with stem cells 3. Participation in another clinical trial within 3 months prior to entry in this trial 4. Any lymphoproliferative disease 5. Tracheostomy, gastrostomy or both 6. Haemophilia, haemorrhagic diathesis or current anticoagulation therapy 7. Known hypersensitivity to bovine foetal whey or gentamicin 8. "Medical precedents" of HIV infection or any serious immunocompromised condition 9. Positive HBV or HCV serology 10. Levels of creatinine in whey > 3.0 in participants not undergoing haemodialysis
Interventions	**Treatment arms** • Intravenous placebo • Intravenous 1 million autologous MSC • Intravenous 2 million autologous MSC • Intravenous 4 million autologous MSC
Outcomes	**Primary outcome measures** 1. Number of serious unexpected adverse reactions attributable to the treatment 2. Infusion site complications 3. Appearance of a new neurological sign or symptom not attributable to the natural progression of ALS **Secondary outcome measures** 1. Change in disease progression 2. Change in muscular force 3. Change in FVC 4. Change in muscular mass estimated by nuclear magnetic resonance imaging of the upper and low extremities 5. Change in neurophysiological parameters 6. Change in quality of life 7. Need for and time to tracheotomy or permanent assisted ventilation
Starting date	July 2014 (duration: 62 months)
Contact information	Fernández O (oscar.fernandez.sspa@juntadeandalucia.es)
Notes	

ALS: amyotrophic lateral sclerosis
ALSFRS: Amyotrophic Lateral Sclerosis Functional Rating Scale
ALT: alanine transaminase
AST: aspartate transaminase
BM-MNC: bone marrow mononuclear cells
FVC: forced vital capacity
HBV: hepatitis B virus
HCV: hepatitis C virus
HIV: human immunodeficiency virus
MRC: Medical Research Council
MSC: mesenchymal stem cells

MSC-NTF: mesenchymal stem cells secreting neurotrophic factors

DATA AND ANALYSES

This review has no analyses.

APPENDICES

Appendix 1. CNMDG (CRS) search strategy

#1 MeSH DESCRIPTOR Motor Neuron Disease Explode All [REFERENCE] [STANDARD]
#2 “moto? neuron? disease?” or “moto?neuron? disease?” [REFERENCE] [STANDARD]
#3 “charcot disease” [REFERENCE] [STANDARD]
#4 “amyotrophic lateral sclerosis” [REFERENCE] [STANDARD]
#5 als:ti or als:ab or nmd:ti or mnd:ab [REFERENCE] [STANDARD]
#6 #1 or #2 or #3 or #4 or #5 [REFERENCE] [STANDARD]
#7 mononuclear NEAR2 leukocyte* [REFERENCE] [STANDARD]
#8 MeSH DESCRIPTOR Stem Cells Explode All [REFERENCE] [STANDARD]
#9 “stem cell*” [REFERENCE] [STANDARD]
#10 MeSH DESCRIPTOR Stem Cell Transplantation Explode All [REFERENCE] [STANDARD]
#11 “bone marrow” [REFERENCE] [STANDARD]
#12 mesenchymal NEAR cell* [REFERENCE] [STANDARD]
#13 mononuclear NEAR cell* [REFERENCE] [STANDARD]
#14 angiogenesis NEAR therap* [REFERENCE] [STANDARD]
#15 #7 or #8 or #9 or #10 or #11 or #12 or #13 [REFERENCE] [STANDARD]
#16 #6 and #15 [REFERENCE] [STANDARD]
#17 (#6 and #15) AND (INREGISTER) [REFERENCE] [STANDARD]

Appendix 2. CENTRAL (CRSO) search strategy

#1MESH DESCRIPTOR Motor Neuron Disease EXPLODE ALL TREES
#2(“motor neuron disease” OR “motor neurone disease” OR “motoneuron disease” OR “motoneurone disease” OR “amyotrophic lateral sclerosis”):TI,AB,KY
#3#1 or #2
#4(mononuclear NEAR2 leukocyte*):TI,AB,KY
#5MESH DESCRIPTOR Stem Cells EXPLODE ALL TREES
#6(“stem cell”):TI,AB,KY
#7MESH DESCRIPTOR Stem Cell Transplantation EXPLODE ALL TREES
#8(“bone marrow”):TI,AB,KY
#9(mesenchymal NEAR cell*):TI,AB,KY
#10(mononuclear NEAR cell*):TI,AB,KY
#11(angiogenesis NEAR therap*):TI,AB,KY
#12#4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11
#13#3 and #12

Appendix 3. MEDLINE (OvidSP) search strategy

Ovid MEDLINE(R) 1946 to June Week 2 2016

Database: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to Present>
Search Strategy:
---
1 randomized controlled trial.pt. (421554)
2 controlled clinical trial.pt. (91049)
3 randomized.ab. (359674)
4 placebo.ab. (174067)
5 drug therapy.fs. (1875235)
6 randomly.ab. (257325)
7 trial.ab. (371730)
8 groups.ab. (1604294)
9 or/1-8 (3826128)
10 exp animals/ not humans.sh. (4266189)
11 9 not 10 (3299247)
12 exp Motor Neuron Disease/ (22274)
13 (moto$1 neuron$1 disease$1 or moto?neuron$1 disease).mp. (7448)
14 ((Lou Gehrig$1 adj5 syndrome$1) or (Lou Gehrig$1 adj5 disease)).mp. (151)
15 charcot disease.tw. (19)
16 Amyotrophic Lateral Sclerosis.mp. (20695)
17 or/12-16 (29255)
18 Leukocytes, Mononuclear/ (31405)
19 Mesenchymal Stromal Cells/ (22707)
20 Bone Marrow Transplantation/ (43703)
21 exp stem cells/ (166583)
22 exp Stem Cell Transplantation/ (64004)
23 (mononuclear adj5 cell$1).tw. (74089)
24 mesenchymal stem cell$1.tw. (26649)
25 (angiogenesis adj3 therap$).tw. (2768)
26 bone marrow.tw. (183315)
27 stem cells.tw. (120452)
28 or/18-27 (470778)
29 11 and 17 and 28 (123)
30 remove duplicates from 29 (116)

Appendix 4. Embase (OvidSP) search strategy

Database: Embase <1980 to 2016 Week 25>
Search Strategy:
---
1 crossover-procedure.sh. (47511)
2 double-blind procedure.sh. (129198)
3 single-blind procedure.sh. (22305)
4 randomized controlled trial.sh. (407169)
5 (random$ or crossover$ or cross over$ or placebo$ or (doubl$ adj blind$) or allocat$).tw,ot. (1266818)
6 trial.ti. (201814)
7 controlled clinical trial/ (393954)
8 or/1-7 (1524261)
9 exp animal/ or exp invertebrate/ or animal.hw. or non human/ or nonhuman/ (22808508)

10 human/ or human cell/ or human tissue/ or normal human/ (17242220)
11 9 not 10 (5599029)
12 8 not 11 (1354806)
13 limit 12 to embase (1117190)
14 motor neuron disease/ or amyotrophic lateral sclerosis/ (32910)
15 (moto$1 neuron$1 disease$1 or moto?neuron$1 disease$1).mp. (11038)
16 ((Lou Gehrig$1 adj5 syndrome$1) or (Lou Gehrig$1 adj5 disease)).mp. (176)
17 charcot disease.tw. (24)
18 amyotrophic lateral sclerosis.tw. (21486)
19 or/14-18 (36431)
20 mononuclear cell/ (41361)
21 exp mesenchyme cell/ (53324)
22 exp bone marrow transplantation/ (58556)
23 exp stem cell/ (257311)
24 exp stem cell transplantation/ (109548)
25 (mononuclear adj5 cell$1).tw. (90921)
26 mesenchymal stem cell$1.tw. (37557)
27 (angiogenesis adj3 therap$).tw. (3566)
28 bone marrow.tw. (238711)
29 stem cell$1.tw. (262770)
30 or/20-29 (653659)
31 13 and 19 and 30 (68)
32 remove duplicates from 31 (68)

Appendix 5. Trials registries search strategies

ClinicalTrials.gov basic search

(motor neuron disease OR amyotrophic lateral sclerosis) AND (cell based OR leukocytes OR mesenchymal OR mesenchym OR mononuclear OR bone marrow OR stem cell OR angiogenesis)

WHO ICTRP advanced search

motor neuron disease AND cell-based OR motor neuron disease AND leukocytes OR motor neuron disease AND mesenchymal OR motor neuron disease AND mesenchym OR motor neuron disease AND mononuclear OR motor neuron disease AND bone marrow OR motor neuron disease AND stem cell OR motor neuron disease AND angiogenesis OR amyotrophic lateral sclerosis AND cell-based OR amyotrophic lateral sclerosis AND leukocytes OR amyotrophic lateral sclerosis AND mesenchymal OR amyotrophic lateral sclerosis AND mesenchym OR amyotrophic lateral sclerosis AND mononuclear OR amyotrophic lateral sclerosis AND bone marrow OR amyotrophic lateral sclerosis AND stem cell OR amyotrophic lateral sclerosis AND angiogenesis.

Appendix 6. Planned methods for data extraction and management

Data extraction and management

Had we identified any eligible studies, we would have used a data extraction form for study characteristics and outcome data which would have first been piloted on at least one study in the review. Two review authors (SFAW and ZKL) would have extracted the following study characteristics from included studies:

1. Methods: study design, date and duration, details of any 'run-in' period (time in a study before participants receive treatment), number of study centres and location, setting, withdrawals.
2. Participants: number, age (mean or median age, range), gender, disease severity, diagnostic criteria, inclusion and exclusion criteria.
3. Interventions: intervention and co-intervention and comparison.
4. Outcomes: primary and secondary outcomes specified and collected, and time points reported.
5. Notes: date trial conducted, funding for trial, notable conflicts of interest of trial authors.

For any included study, we would have undertaken the following steps: i) two review authors (SFAW and ZKL) independently extract the outcome data and note in the 'Characteristics of included studies' table if outcome data are not reported in a usable way, with disagreements resolved by consensus or by involving another author (RAA); ii) one review author (NAI) transfers data into Review Manager 5 (RevMan 5) software (RevMan 2014), one review author (SFAW) checks the outcome data entries, and another review author (NML) spot-checks study characteristics for accuracy against the trial report.
If reports had required translation, the authors would have extracted data from the translation provided, with data cross-checked against the original report if possible.

Assessment of risk of bias in included studies

We planned to have two review authors (NML and SFAW) independently assessing the risk of bias for each study according to the domains listed below, as outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011), with resolution of disagreement by discussion or by involving another author (RAA).

1. Random sequence generation.
2. Allocation concealment.
3. Blinding of participants and personnel.
4. Blinding of outcome assessment.
5. Incomplete outcome data.
6. Selective outcome reporting.
7. Other bias, such as premature termination and extreme baseline imbalance.

We planned to accord a judgment of low or high risk of bias if there was sufficient information in the study report, and justify our grade with a quote from the study in the 'Risk of bias' table. If there was insufficient information available from the study to enable a judgment, we would have graded the risk of bias as unclear. We planned to consider blinding separately for clinical and laboratory outcomes where necessary. Where information on risk of bias related to unpublished data or correspondence with the study authors, we would have noted this in the 'Risk of bias' table.

Measures of treatment effect

Had there been any included study, we would have analysed dichotomous data as risk ratios and continuous data as mean differences, or standardised mean differences if conceptually similar outcomes were measured on different scales. In this case, we would have adjusted all the scales to achieve a consistent direction of effect.
We planned to undertake meta-analyses only where the participants, intervention, comparison and outcomes were similar enough for pooling to be meaningful, and only narratively describe skewed data reported as medians and interquartile ranges.

Unit of analysis issues

For cluster RCTs (in other words, trials in which the assignment to intervention or control group was made at the level of the unit/ward rather than the individual participant), we planned to assess whether the study authors had made appropriate adjustments for the effects of clustering, using appropriate analysis models such as the Generalized Estimating Equation model. We would have inspected the width of the standard error (SE) or 95% confidence interval of the estimated treatment effects to double-check the possible unit of analysis in the study. If we found an inappropriately small SE or a narrow 95% CI, we would have asked the authors of the study to confirm the unit of analysis.
If no adjustment was made for the effects of clustering, we would have performed adjustments by multiplying the SEs of the final effect estimates by the square root of the 'design effect', represented by the formula, $1 + (M-1) \times ICC$, where M is the average cluster size (number of participants per cluster) and ICC is the intracluster correlation. The average cluster size (M) from each trial would be determined by dividing the total number of participants by the total number of clusters. We planned to use an assumed ICC of 0.10, as we consider this to be a realistic general estimate that is derived from previous studies on implementation research (Campbell 2001). We would have combined the adjusted final effect estimates from each trial with their SEs in meta-analysis using generic inverse variance methods, as stated in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).
If the determination of the unit of analysis was not possible, we planned to include the studies concerned in a meta-analysis using the effect estimates reported by the authors. We would also have performed sensitivity analyses to assess how the overall results were affected by the removal of the studies in which i) adjustment of unit of analysis was appropriate but not possible and ii) the unit of analysis was unknown.

Dealing with missing data

If key information were missing, such as study characteristics, methods or outcome data, we planned to contact investigators to obtain the relevant information. Where this was not possible, we would have conducted a deterministic sensitivity analysis at the study level by adopting the 'worst case scenario' approach using the major outcomes with sufficient data, for instance, our primary outcome of change in functional rating scales, such as the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) or Expanded Disability Status Scale (EDSS) measured at 6 months. If the effect estimate of the study changed substantially following our sensitivity analysis, we would have considered the study to be at high risk of attrition bias. At the review level, we would again have conducted a sensitivity analysis to explore the impact of including such studies with high risk of attrition bias in the overall pooled estimates of the major outcomes.

Assessment of heterogeneity

We planned to use the I^2 statistic to measure heterogeneity among the trials in each analysis. If we had identified substantial unexplained heterogeneity (as shown by an I^2 of greater than 50%) we would have explored possible causes by prespecified subgroup analyses (see Subgroup analysis and investigation of heterogeneity).

Assessment of reporting biases

If we had been able to pool more than 10 trials, we would have created a funnel plot to explore possible publication biases. If we had found significant asymmetry in the funnel plot, which might indicate possible publication bias, we would have reported this with a note of caution in the discussion, taking into account the area of the void in the funnel plot. We did not plan to further explore publication bias using statistical methods in view of the limitations of these methods in the presence of the relatively small number of studies in a typical systematic review (Higgins 2011).

Data synthesis

We planned to perform our meta-analysis in Review Manager 5 (RevMan 2014), using a fixed-effect model. We planned to perform a sensitivity analysis to assess the change in the overall results with a random-effects model.

'Summary of findings' table

Had there been included studies with important outcomes reported, we would have created a 'Summary of findings' (SOF) table comparing cell-based therapy versus placebo or no additional treatment using the following outcomes: ALSFRS (at 6 and 12 months), manual muscle testing (at 6 months), FVC (at 6 months), survival rate (at 12 months), and adverse events (at any given time point). Our judgment on the overall quality of the body of evidence would have been guided by the five GRADE considerations, namely limitations in study design, consistency of effect, imprecision, indirectness and publication bias. We planned to use methods and recommendations described in Chapter 12 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011) using the GRADE profiler (GRADEpro) software (GRADEpro 2014). We planned to justify all decisions to down- or up-grade the quality of studies using footnotes in the SOF table.

Subgroup analysis and investigation of heterogeneity

We planned to carry out a subgroup analysis based on the type of cell-based therapy received, i.e. either BM-MNCs, BM-MSCs, M-PBMNCs, OESCs or NSCs. We would also have conducted a subgroup analysis based on delivery method, i.e. intrathecal, intracranial, intraspinal and intravenous and for the primary endpoint that was measured at two separate time points. We would have used functional scales, such as the EDSS and ALSFRS, FVC, quality of life scores, MRI changes, survival rate, neurophysiological index and adverse events as outcome measures.
We planned to use the formal test for subgroup interactions in Review Manager 5 (Higgins 2011).

Sensitivity analysis

We planned to carry out the following sensitivity analyses if there were sufficient studies included:

1. Repeat the analysis excluding studies at high risk of selection and attrition biases.
2. Repeat the analysis excluding large studies to assess the effect of these studies on the overall results.
3. Repeat the analysis with a random-effects model.
4. Repeat the analysis excluding unpublished studies.

If the overall results were affected substantially by the sensitivity analysis, we would have placed a note of caution in our discussion and conclusions regarding the certainty of our estimates, and proposed a need for further research where appropriate to explore the possible sources of variation in the outcome estimates.

CONTRIBUTIONS OF AUTHORS

SFAW, ZKL, NML and NAI wrote the review and approved the review in its final form.

SFAW designed the project and review.

SFAW, ZKL and NAI drafted the search strategy.

SFAW, ZKL and NAI performed the study screening and selection.

NML and RAA reviewed the analysis of the search

DECLARATIONS OF INTEREST

SFAW: none known

RAA: none known

NAI: none known

NML: none known

ZKL: none known

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- No sources of support supplied, Malaysia.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In our protocol, we did not specifically state the other sources that we handsearched. In the current review, we handsearched publications from the following journals: Cytotherapy (January 1999 to 21 June 2016), Cell Transplantation (issue 1, 2001 to issue 6, 2016), Cell Stem Cell (issue 1, 2007 to issue 6, 2016) and Stem Cells (issue 1, 1993 to issue 6, 2016) for relevant articles.