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The potential of stem cell therapy in Multiple Sclerosis treatment: a review

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Abstract

Multiple Sclerosis is an autoimmune and neurodegenerative disease of the central nervous system. There are currently 2.8 million people living with Multiple Sclerosis worldwide, including 9000 people in Ireland, with a prevalence of 193 per 100,000 people. Symptoms of Multiple Sclerosis include sensory loss, fatigue, cognitive dysfunction, spasticity, depression, optic neuritis, gait ataxia, diplopia, and loss of bladder control. Currently, there is no standardised treatment or cure for Multiple Sclerosis with many strategies focusing on symptoms. Stem cells have emerged as promising approaches for Multiple Sclerosis therapeutics. Clinical trials primarily focus on mesenchymal stem cell-based therapies for Multiple Sclerosis. Furthermore, due to their immunomodulatory and anti-inflammatory characteristics, adipose derived stem cells have recently surfaced as novel therapies for diseases, such as Multiple Sclerosis. Current knowledge shows that there is potential for adipose derived stem cells to replace other adult stem cell-based therapies, as they are much more readily available and involve less invasive isolation procedures. There are currently 371 clinical trials involving mesenchymal stem cells and 462 clinical trials involving adipose derived stem cells, with research focused on skeletal applications, wound healing, and to a lesser degree, haematological and neurological disorders. Although adipose derived stem cells could represent a valuable treatment option for many diseases, several drawbacks to their advancement remain, such as their pro-tumorigenic properties, possible negative alteration of the immune system, and the difficulty in assessing their migration patterns once administered in vivo. Adipose derived stem cell-based therapies have been described as a double-edged sword, with both beneficiary and unfavourable effects observed. Most current clinical trials continue to focus on establishing the safety and efficacy of adipose derived stem cell-based therapeutics, with promising results becoming progressively evident. This review reports stem cell-based therapies as potential therapeutic approaches in the treatment and management of Multiple Sclerosis. This paper explores the characterisation and aetiology of the disease, current treatments for Multiple Sclerosis, and clinical trial data that investigated the therapeutic safety and efficacy of proposed stem cell-derived Multiple Sclerosis treatments.

Keywords: Multiple Sclerosis, MS, MSC, ADSC, stem cell therapies.

1. Introduction

Multiple Sclerosis (MS) is autoimmune and neurodegenerative disorder (Bowles *et al*., 2016) of the central nervous system (CNS), characterized by an autoimmune reaction in which there is an uncontrolled immune response to myelin proteins resulting in motor dysfunction and cognitive decline (Bejargafshe *et al*., 2019; Ksiazek-Winiarek *et al*., 2015). MS is the most common chronic neurological disease among young adults both in Europe and North America with symptoms ranging from severe fatigue and depression to sensory loss and cognitive impairment (Wallin *et al*., 2019; Zasadzka *et al*., 2021). There are currently 2.8 million people living with MS worldwide, representing 193 per 100,000 people, with 30% males and 70% females globally affected. In Ireland, approximately 9,000 people (25% male and 75% female) are living with MS, with an average age of diagnosis of 32 in males and 37 in females (MS International Federation, 2022).

There are four main types of MS based on the degree of progression (**Table 1**) where relapsing remitting MS **(**RRMS) is the most common, and progressive relapsing MS (PRMS) is the least common. Primary progressive MS (PPMS) and secondary progressive MS (SPMS) stages occur in between RRMS and PRMS stages (Bejargafshe *et al*., 2019; Ghasemi *et al*., 2017).

Table 1: The four phenotypes of Multiple Sclerosis (Klineova and Lublin, 2018).

Relapsing remitting MS: RRMS	**Primary progressive MS: PPMS**	**Secondary progressive MS: SPMS**	**Progressive relapsing MS: PRMS**
Relapses with recovery and stable course between relapses	Gradual neurologic deterioration from onset of symptoms with no relapses and no remissions	Gradual neurologic deterioration and worsening of symptoms with or without relapses and remissions	Gradual neurologic deterioration from onset of symptoms with relapses but no remissions

MS is characterised based on the potential for relapse disease progression, recovery, and remission. This can range from relapses with recovery (RRMS), to gradual neurologic deterioration with and without relapses (SPMS, PPMS and PPMS, respectively) and without the possibility of remission (PPMS, SPMS and PRMS) (Dhaiban *et al*., 2020). Principal pathological features of MS include inflammation, demyelination, and loss or impairment of axons, as shown in **Figure 1**.

The aetiology of MS is still largely unknown, but it has been suggested that environmental factors such as vitamin D and B12 deficiencies, Epstein-Barr viral infection, cigarette smoking, mycoplasma pneumonia, human herpes virus type six, and possibly obesity may play a role (Ghasemi *et al.*, 2017; Richter *et al*., 2017; Stankov and Stepancev, 2018). Furthermore, several genetic factors have been linked to the disease including over 500 genes and more than 200 genetic variants. Gene variants (e.g., the allele DRB1*15:01) located in the human leukocyte antigen (HLA) complex were shown to have the strongest associations with MS risk as well as alleles of interleukin 2 receptor alpha (IL2RA) and interleukin 7 receptor alpha (IL7RA) (Garg and Smith, 2015; Groen *et al*., 2020; Hedström *et al*., 2021). The level of shared genetic information between a patient and their family members is significant with the risk rate of monozygotic twins (100% genetic similarity) being approximately 25% (Ghasemi *et al*., 2017).

Thus, it is improbable that only one risk factor is associated with the onset of MS, but rather a combination of different factors, such as genetic susceptibility, viral infections, vitamin deficiencies, and environmental factors through various mechanisms (Groen *et al*., 2020).

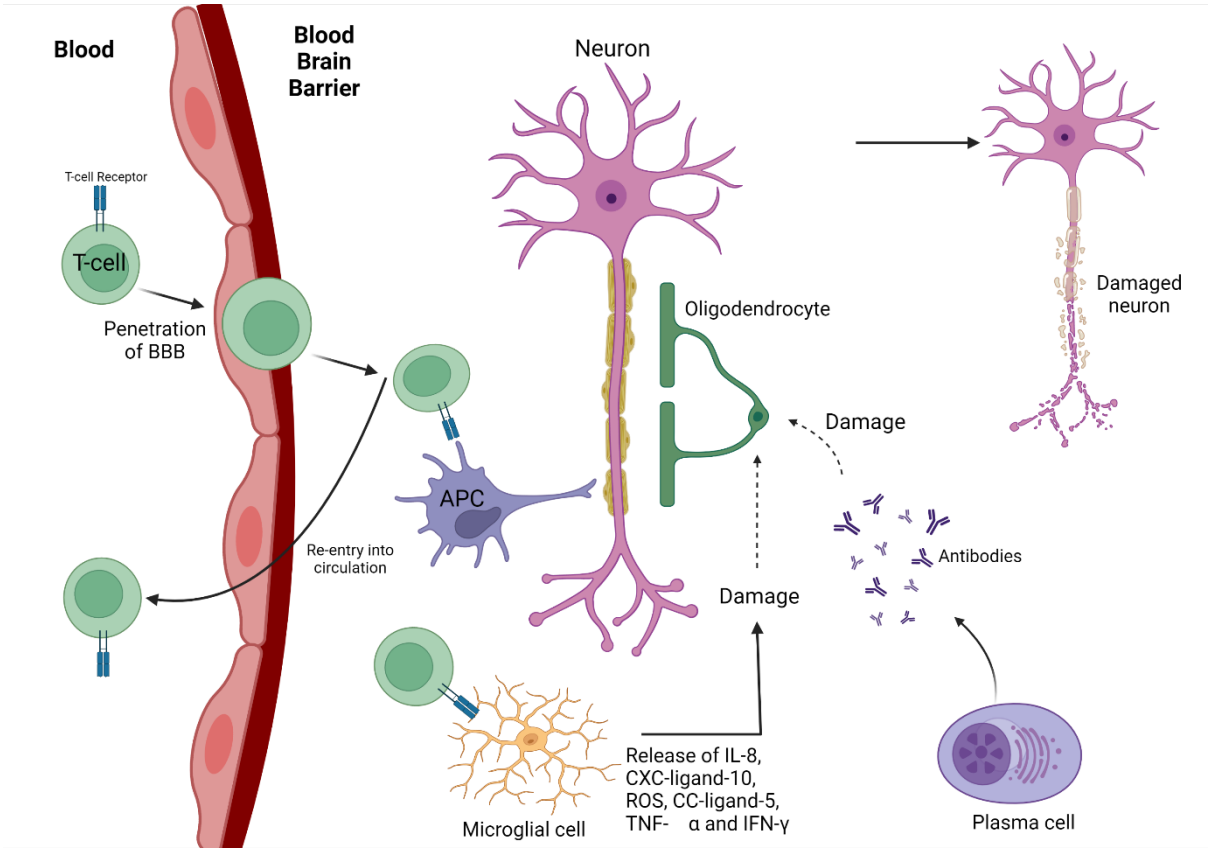

Figure 1: The pathophysiology of Multiple Sclerosis. The myelin-sensitive T-cell becomes activated in the blood and breaches the blood brain barrier where it triggers the recruitment of inflammatory cells and cytokines. These inflammatory mediators cause damage and demyelination of oligodendrocytes resulting in plague formation around the neuron, rendering it damaged. Damaged neurons lead to loss of neurological and motor function as seen in MS (Amarculesei *et al*., 2023).

Stem cells are multipotent cells that can be isolated from four main tissue sources; embryonic (Embryonic Stem Cells), foetal (Foetal Stem Cells), adult (Adult Stem Cells) and somatic (induced Pluripotent Stem Cells) (**Figure 2**) (Bacakova *et al.*, 2018). Stem cells are fast emerging as therapeutic strategies for numerous diseases, such as neurodegenerative diseases, autoimmune disorders, inflammatory diseases and cancer (Lu *et al*., 2022; Regmi *et al.*, 2019; Vissers *et al.*, 2019; Zhao *et al.*, 2020). Mesenchymal Stem Cells (MSCs) are non-haematopoietic stromal cells that have been used in research and clinical therapies over the last 30 years, with the most common sources of MSCs being bone marrow and adipose tissue (Pittenger *et al.*, 2019). MSCs are capable of self-renewal and multi-lineage differentiation into several different cell types (Vandana *et al*., 2021). Regulations for stem cell use varies across Europe, with each country having its own guidelines on the topic. There are currently no regulations for stem cell research in Ireland (Small, 2022; Staunton, 2018). However, the

landscape of stem cells for human use is evolving, with new research and centres of excellence being established in Ireland in recent years, such as The Centre for Cell Manufacturing Ireland at University of Galway who were recently granted a licence from the Irish Medicines Board for the production of stem cells intended for use in humans. Adipose-derived mesenchymal stem cells (ADSCs) are emerging as a promising and reliable adult stem cell source due to their differentiation capabilities, their abundance in the human body, and their relatively non-invasive collection method (Gimble *et al.*, 2007; Zhang *et al.*, 2015).

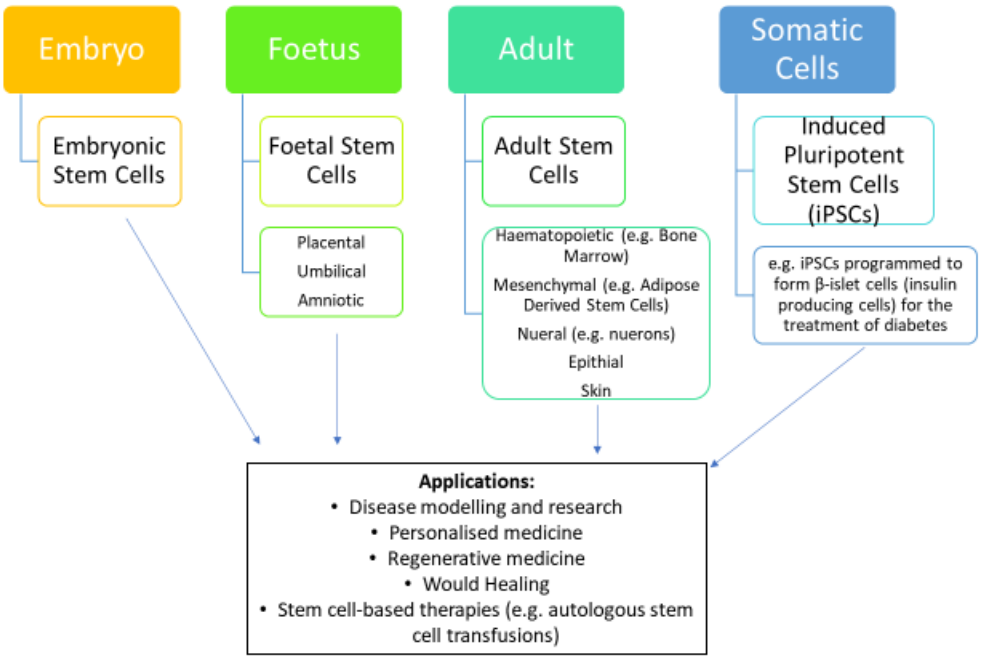

Figure 2: Schematic of stem cell sources and types (Amarculesei *et al*., 2023). There are four main sources of stem cells: embryonic tissues, foetal tissues, adult tissues, and somatic tissues. Embryonic Stem Cells are isolated from embryonic tissues. Foetal Stem Cells are isolated from foetal tissues in the form of placental, umbilical, and amniotic stem cells. Adult Stem Cells are isolated for several types of human tissues and can be categorised as Haematopoietic (e.g., Bone Marrow Stem Cells), Mesenchymal (e.g., Adipose Derived Stem Cells), Neural, Epithelial and Skin Stem cells. Induced Pluripotent Stem Cells are programmed somatic cells and can be designed to conform to desired cell types, e.g., Beta (β)-islet cells (insulin producing cells) for the treatment of diabetes. These categories of stem cells have many applications such as disease modelling and research, personalised and regenerative medicine, wound healing and stem-cell based therapies.

2. Current Multiple Sclerosis therapies

Currently there is no definitive cure for MS, as it involves a multitude of factors including axon-degenerative, inflammatory, and myelin-degenerative (Ghasemi *et al*., 2017; Piehl, 2021). Moreover, MS is progressively characterised by both acute and chronic episodes (Ghasemi *et al*., 2017; Piehl, 2021). First-line treatments include interferon beta (IFN-β) and glatiramer acetate (GA). The former is an immunomodulator, that interferes with virus replication, while the latter exhibits multiple immunomodulatory actions such as anti-inflammatory and

immunosuppressive effects on T helper (Th) cells. Although these drugs can lessen symptoms, IFN-β and GA both frequently fail to show satisfactory clinical effectiveness, often with serious side effects such as cardiac toxicity, as well as having minute effects in the progressive stages of MS (Ghasemi *et al*., 2017; Tuosto, 2015). While some of these treatments have been shown to reduce the frequency of relapse or slow the progression of the disease, these treatments are limited to ameliorate symptoms and chronic inflammation (Stepien *et al*., 2016). There are currently numerous medications for MS, with over thirty drugs controlling disease symptoms including oral therapies (e.g., dimethyl fumarate, teriflunomide, fingolimod, cladribine), injectables (e.g., glatiramer acetate, interferons), and infusion therapies (e.g., ocrelizumab, natalizumab, alemtuzumab) (Fischer *et al*., 2021; Jafarzadeh *et al*., 2019). Two of the most common secondary-line therapeutic drugs include Natalizumab, which is a monoclonal antibody targeting the protein alpha 4 integrin, and Fingolimod, a sphingosine 1-phosphate receptor modulator (Mameli *et al.*, 2016; McGinley and Cohen, 2021). Disease-modulatory therapies (DMTs) are available for the treatment of MS; these anti-inflammatory and immunomodulating agents improve the long-term outcomes of MS and can decrease specific pathological symptoms and/or diminish the progression of the disease (**Table 2**) (Ghasemi *et al*., 2017; Piehl, 2021).

Table 2: Currently approved disease-modulatory therapies in the European Union and the United States for Multiple Sclerosis (Piehl, 2021).

DMT Class	Examples	Potential Side Effects
Cell depleting	Ocrelizumab, Alemtuzumab	Infections, cancer, autoimmune reactions, infusion reactions
Cell migration modulators	Fingolimod, Natalizumab	Infections, hepatic injury, teratogenicity, progressive multifocal leukoencephalopathy
Glatiramer acetate	Glatiramer Acetate	Injection-site lipoatrophy
Interferons	Interferon β-1a	Hepatic injury, depression
Oral immuno modulators	Dimethyl Fumarate, Teriflunomide	Lymphopenia, gastrointestinal disturbances, alopecia, teratogenicity

Current therapeutics, despite having some benefits, do not prevent further neurodegeneration and thus, novel approaches are needed (Ghasemi *et al*., 2017; Kremer *et al*., 2019). Due to the nature of progressive MS, early intervention is paramount in slowing the progression of the disease, with upcoming research finding that differences in patient response to current treatment options may be due to individual disease status and characteristics, such as age (Pozzilli *et al*., 2023). Thus, there is a great need for new, individualised therapeutics which would suppress the autoimmune responses, protect the neurons and the axons against the degeneration process, and improve remyelination in patient cohorts (Stepien *et al*., 2016).

3. Adipose derived stem cells

Since their first isolation and classification in 2001, ADSCs have been one of the most promising stem cell populations identified thus far due to their abundance and availability compared to other stem cells (Chu *et al.*, 2019; Si *et al.*, 2019; Zuk *et al.*, 2001). ADSCs are a subset of MSCs found in the stromal vascular fraction (SVF) which can be isolated through enzymatic processes (Palumbo, *et al*., 2018). Three minimal criteria for the definition of ADSCs were proposed by the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Firstly, the capability for plastic adherence; secondly, expression of CD73, CD90, and CD105, and a lack of expression of HLA-DR, CD11b, CD14, CD19, CD45. Finally, differentiation into a number of different lineages (Palumbo, *et al*., 2018). The transplantation of ADSCs into damaged locations or sites of inflammation, allows for the interaction of ADSCs with the damaged environment. In this microenvironment, ADSCs can secrete cytokines, chemokines, growth factors, and microRNAs (miRNAs). This leads to the generation of new committed progenitor cells, immunomodulation, cell proliferation, anti-apoptosis, regeneration, and angiogenesis (Mazini *et al*., 2021; Miana and Gonzalez, 2018). When exposed to specific environments ADSCs can differentiate into mature adipocytes, chondrocytes, osteoblasts, myocytes, hepatocytes, endothelial cells, neuronal cells, and others (Rajan and Rajesh, 2021).

4. Therapeutic application of adipose derived stem cells in neurodegeneration and neurological disorders

ADSCs have emerged as promising therapeutic approaches in regenerative medicine and tissue engineering due to their "stemness", referring to the ability of stem cells to self-renew and to multi-lineage differentiate (Zhang *et al*., 2015). In recent years, the therapeutic application of MSCs, in particular ADSCs, has grown rapidly due their stemness, low immunogenic profile, migration properties and cellular repair activities (Mazini *et al*., 2021). The development of cell-based therapies for regenerative medicine to treat and potentially eradicate prevalent degenerative diseases has been described as one of the most promising scientific advancements of modern medicine (Aly, 2020). Researchers have recently identified the therapeutic component of MSCs, known as extracellular vehicles (EVs) (Musiał-Wysocka *et al.*, 2019). EVs are secreted by MSCs and facilitate the communication and transfer of biological components (e.g., miRNAs and other functional proteins) between cells (Hu *et al*., 2022).

Previous literature has highlighted the therapeutic potential of ADSC-derived EVs (ADSC-EVs) to treat neurodegenerative diseases (Ding *et al.*, 2022). ADSC-EVs exerted neuroprotective effects in an *in vitro* cell model of amyotrophic lateral sclerosis (ALS) (Bonafede *et al*., 2019). The neuroprotective potential of ADSCs was also observed in *in vitro* and *in vivo* murine models, where motor neuron protection and survival were observed in the presence of ADSCs (Ciervo *et al*., 2021). This was highlighted as a promising therapy for Parkinson's Disease (Li *et al*., 2021). Furthermore, ADSC-EVs modulated gene expression in neurorepair, and improved memory loss in murine models of Alzheimer's disease (Ma *et al*., 2020). ADSCs were shown to supress the neurodegenerative process in murine models of multiple system atrophy by restoring impaired dopamine receptors and pathway and supressing inflammation and apoptosis (Chang *et al*., 2020). In addition, stem cell therapy is also emerging as a promising therapeutic approach in the treatment of autoimmune disorders of the CNS, such as MS (Gugliandolo *et al.*, 2020).

The neuroprotective and immunoregulatory characteristics of MSCs, and more recently ADSCs, have been identified as potential, novel approaches in MS treatment (Barati *et al*., 2020;

Hassanshahi *et al*., 2021; Mansoor *et al*., 2019; Uccelli *et al.*, 2021). ADSCs have shown their ability to differentiate into oligodendrocyte precursor cells which myelinate the axons in the CNS, which in turn, supports axon protection and fast, effective impulse conduction (Bradl and Lassmann, 2010; Williamson and Lyons, 2018). The ability of ADSCs to differentiate into oligodendrocyte precursor cells is a significant finding in the treatment of demyelinating diseases such as MS (Williamson and Lyons, 2018). Research has shown that treatment with ADSCs, in conjunction with the free radical scavenger Edaravone, was effective in improving motor function and histopathological presentation in murine MS models (Bakhtiari *et al*., 2021).

5. Clinical trials: stem cell therapy for multiple sclerosis

Many MSC- and ADSC-based therapies for disease have been studied since the first autologous MSC-based treatment trial by Lazarus *et al*. (1995). Several clinical trials have highlighted the therapeutic potential of allogenic and autologous MSCs (Kabat *et al*., 2020). In addition, since their emergence over 20 years ago, ADSCs have quickly gained interest due to their current and potential use in regenerative medicine (Trzyna and Banas-Zabczyk, 2021). Due to the many advantages that ADSCs have, such as immunomodulatory and anti-inflammatory properties, they have been investigated for the treatment of autoimmune and neurodegenerative diseases (Ceccarelli, *et al*., 2020).

A phase I/II open-safety clinical trial involving 15 patients with MS and 19 patients with ALS focused on the intrathecal and intravenous administration and the feasibility and safety evaluation of autologous bone marrow derived MSC. Twenty-one patients experienced febrile reactions following injection that lasted up to 7 days which were attributed to the lumbar punctures and one patient experienced aseptic meningitis which was most likely caused by residual dimethyl sulfoxide in the culture medium. The clinical effects reported included the gradual decline of Expanded Disability Status Scale (EDSS) score in MS patients and the initial deterioration of Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) score in ALS patients followed by a stable score during the six-month follow-up. Immunological effects were noticed in both MS and ALS. This research described a 72% increase of $CD4^+$ and $CD25^+$ regulatory T cells and a reduction of up to 60% of $CD86^+$, $CD83^+$, $CD40^+$, and HLA-DR^+. These changes indicate that MSCs downregulate activated lymphocytes and antigen presenting cells. No major immediate or late adverse effects were reported during follow-ups in the next 25 months, and MRIs after one year showed no pathology or new disease activity indicating an acceptable short-term safety report of intrathecal injection of MSCs. The study demonstrated that transplantation of MSCs were clinically feasible and relatively safe, they also induced immediate immunomodulatory effects. Nevertheless, further trials were needed to assess the long-term safety and feasibility of MSCs transplantations (Karussis *et al*., 2010). Further trials that investigate the long-term effects of these types of treatments would benefit patients in both early and late stages of MS as MS prognosis vary amongst individuals.

Burt *et al.* (2019) conducted a phase II clinical trial on 103 patients affected by RRMS to assess time to disease progression. 51 patients randomised received DMT (e.g., interferons, natalizumab, dimethyl fumarate) and 52 patients received hematopoietic stem cell transplantation (HSCT) obtained from peripheral blood stem cells. Three patients in the HSCT group and 34 patients in the DMT group displayed disease progression. The HSCT compared to DMT group resulted in prolonged time to disease progression. The HSCT group showed signs of improvement in EDSS, Neurologic Rating Scale (NRS), MS Functional Composite (MSFC) scores, and Magnetic Resonance Imaging (MRI) T2-weighted lesion volume which were higher than those showed in pharmaceutical trials. Furthermore, patients that crossed over from the

DMT to HSCT group showed improvements in EDSS scores. Adverse events did not include potential life-threatening events (e.g., embolism, sepsis, surgery, myocardial infarction) but rather metabolism abnormalities, hypertension, elevated transaminases in the liver, respiratory tract infections, urinary tract infections, and dermatomal varicella zoster reactivations. Overall, the DMT group had a higher rate of infection than the HSCT group. Further studies are needed to assess the long-term safety of HSCT and to replicate the findings of the trial (Burt *et al*., 2019). Following this assessment, there was an inherent need to assess the safety and efficacy of MSCs in MS clinical trials. This study concluded that disease progression was slowed following treatment with haematopoietic stem cells compared to disease-modifying therapy. Further research was recommended to assess safety and trial results over a longer study period. This trial demonstrated a slowing of disease progression, which is a significant factor in progressive neurodegenerative diseases like MS, where disease progression is proportional to adverse symptoms and disease presentation in affected individuals. Similarly to conclusions drawn by Karussis *et al*. (2010), assessing the long-term effects of these therapies for MS would benefit a wider cohort of patients with both early- and late-stage MS.

In 2022, a double-blind, phase II clinical trial involving 28 patients affected by MS was performed to assess cognition before and after MSC therapy as well as their safety and feasibility. Patients were randomly assigned to an MSC treatment group and a placebo group. At week 24 the first group switched to placebo while the second group switched to treatment. Although the results showed no detectable effects and no evidence of neural repair possibly due to the short term of the study (48 weeks) and lack of a placebo group. This study concluded that the MSC therapy did not improve cognition, but the treatment did not have an adverse effect proving the safety and feasibility of MSC-based therapies. Therefore, MSC therapy was demonstrated to be safe and feasible with little negative cognitive effects. However, Berard *et al.* (2022) highlighted that further long-term studies with control group are needed to assess the potential cognitive benefits and long-term changes that MSC might have (Berard *et al*, 2022). Establishing the safety and feasibility of MSC-based therapies in clinical trials is one step closer to stem cell therapies for MS treatment.

Recently, Tremblay *et al* (2022) conducted a randomized, placebo controlled, crossover, double-blind phase II clinical trial involving twenty patients affected by MS, to examine the safety, feasibility, and potential of MSCs. Of the 20 patients, 9 received MSCs intravenously and 11 received a placebo. At week 24, groups which received MSC treatments switched to the placebo group and vice versa. Various parameters were evaluated including alterations in hand performance, disability status, corticomotor excitability, intra- and inter-hemispheric inhibition, and motor conduction at 0, 24 and 48 weeks. The study showed no neurophysiological improvements and no signs of halting disease progression. Possible explanations for the lack of positive results were suggested to be the heterogeneity of MSC production, MSC bioavailability upon injection, little understanding of the impact on the patient, variations in injection devices, retention, and survival rate of MSCs, variations of diseases severity between patients, and immune response as well as cytotoxicity in response to MSC infusions (Tremblay *et al*, 2022). This study concluded that no neurophysiological improvements were observed in patients with MS following autologous MSC infusion, meaning that MSC therapy did not have a therapeutic benefit in improving these disease precipitations of MS (Tremblay *et al*, 2022). MSCs have previously been described as a double-edged sword and this study highlights the need for rigorous safety strategies when dealing with MSC clinical trials (Levy *et al*., 2020; Norozi *et al*., 2016; Yao *et al*., 2013). Future studies should focus on multiple infusions strategies, taking into consideration the differences in disease severity between patients, the micro-environment of the disease, and allowing more time after infusions. Concurrently, ADSCs were coming to

the forefront of MSC research with the commencement of clinical trials into potential ADSC-based treatments for MS.

It should be noted that a phase I/II clinical trial was performed in 2018 to assess the safety of autologous ADSCs infusion and feasibility on 30 patients affected by SPMS where 11 where given placebo and 19 were infused with ADSCs. Different parameters were assessed such as number of exacerbations, EDSS score, immunological tests, MRI (T lesions), cerebrospinal fluid analysis, evoked potentials, cognition tests, and quality-of-life tests. Following intravenous infusion of autologous ADSCs, patients were assessed at 30 days, 6 months, and 12 months. No clear effects were detected, although some non-statistically significant post-baseline changes were noticed in the MRI studies and in the evoked potential studies. A possible explanation for these minimal effects may be the already advanced and prominent neurodegenerative phase of disease of the patients. The most common adverse effects reported were urinary and respiratory infections, and anemia. Two patients from the placebo group died, one due to bronchial aspiration while feeding and another due to respiratory infection nine months after infusion, however, these deaths were not attributed to the treatment investigated. This clinical trial demonstrated the safety and feasibility of ADSCs infusion and recommended that larger studies should focus on the therapeutic potential of ADSCs in the future (Fernandez *et al.*, 2018).

The following year, Duma *et al*., (2019) conducted a phase I clinical trial to assess the safety, feasibility, and potential disease improvement of autologous adipose-derived stromal vascular fraction (ADSVF) on 31 patients affected by a variety of neurodegenerative conditions such as Alzheimer's disease, ALS, Parkinson's disease, traumatic brain injury, stroke, spinal cord injury, and progressive MS. This trial assessed patients over a 3-year period compared to the 12-month trial conducted by Fernandez *et al*. (2018). Follow-up ranged from 2 to 36 months and results showed 87.5% disease stability or improvement in Alzheimer's disease and progressive MS. One Alzheimer's patient in particular showed signs of hippocampal volume increase while one progressive MS patient, which was previously wheelchair bound, improved the patients transition to using a walker and driving. Some patients showed a decline after four to six weeks suggesting the need for multiple injections over time. Furthermore, 6 patients died due to natural disease progression suggesting that earlier intervention might be beneficial, and 11% of the patients suffered signs of meningismus and later recovered. No serious adverse effects were observed in participants, with some clinical improvements being observed. As a result, this phase I trial was presented to the FDA for approval to continue with a Phase II trial to address innate patient variability of injected cell quality and number (Duma *et al*., 2019). This study concluded that ADSVF treatments were safely injected with no serious adverse effects, with some clinical improvements being observed. Duma *et al*., (2019) recommended that Phase II clinical trials are necessary to confirm these findings. Similarly to Fernandez *et al.* (2018), Duma *et al*. (2019) reiterated the safety and feasibility of ADSC profile of ADSC infusions, and advanced this finding to include the observation of clinical improvements in treated individuals. These continuous advancements in stem cell technology and clinical trial outcomes are expanding the repertoire of current treatment options, inherently improving patient care and prognosis.

ADSC-based therapies have the potential to replace other MSC-based technologies. As well as having the same properties than its comparable stem cell counterparts, ADSC-based therapies have a less invasive isolation procedure that other MSCs (Mazini, *et al*., 2019). The therapeutic properties of ADSCs in treating MS have proven effective in past clinical trials, with further exploration required so as ADSC-based treatments for MS can be fully advanced (Berard *et al*., 2022; Burt *et al.*, 2019; Fassas *et al*., 2002; Tremblay *et al*., 2022). The future of ADSC-based

therapies for MS proves promising, as ADSC-based clinical trials have investigated the benefit of this approach in other autoimmune and neurodegenerative disorders. There is a need for further research and clinical trials to investigate the potential of ADSCs as novel treatments for MS, especially as ADSC-based transplantation has shown notable improvements in animal models of MS (Hedayatpour *et al.*, 2013; Shalaby *et al.*, 2016).

6. Adipose derived stem cell therapies for MS

Safety and efficacy of ASDC-based MS therapies

Most of the clinical trials mentioned focused on assessing the safety and feasibility of MSCs and ADSCs as potential therapeutics for MS and other neurodegenerative conditions. Karussis *et al.,* (2010) conducted a clinical trial on MS patients involving BM-derived MSCs and the results showed no major adverse effects indicating the short-term safety of the infusions, further trials were recommended to assess the long-term safety of MSCs transplantations. On the other hand, Burt *et al*., (2019), concentrated on assessing the time to disease progression between a DMT groups and a HSCT group of RRMS patients, and the results showed improvements in the HSCT. Although many patients were affected by some adverse effects, such as respiratory and urinary tract infections, these were not potential life-threatening events and therefore suggested the safety of MSCs in the short-term. It was suggested that future studies should replicate the results of the trail and that further analysis is needed to assess the long-term safety of HSCT. Another clinical trial by Berard *et al*., (2022) involving the infusion of MSCs in MS patients proved the short-term safety and feasibility of MSCs with minimal cognitive adverse effects, suggesting long-term studies in the future with control groups. Although most of the trials discussed above showed improvement after MSCs infusion, the trial conducted by Tremblay *et al.*, (2022) showed no improvements neurophysiologically and no halting of disease progression suggesting that future studies should focus on the micro-environment of the disease and different disease severity between patients as well as longer term trials. When focusing on ADSCs, Fernandez *et al.*, (2018), conducted a trial on SPMS patients with the primary focus on assessing their safety and feasibility; the results showed minimal changes in MRIs and evoked potential studies, as well as no major adverse effects with common adverse effects such as anaemia, respiratory and urinary infections. It was suggested that larger studies should be performed to confirm the safety and feasibility of ADSCs. Finally, Duma *et al.*, (2019) focused on the infusion of ADSVF on patients affected by a variety of neurodegenerative diseases; the results were promising for MS and Alzheimer's patients showing improvements or disease stability, as well as being safe and feasible with only 11% of the patients suffering from signs of meningismus which recovered quickly after.

Overall, the clinical trials demonstrated the relative safety and feasibility of MSCs and ADSCs, with no major life-threatening adverse effects and with the most common adverse effects being urinary and respiratory infections. Most studies suggested longer term studies to assess the long-term safety of MSCs and their potential as therapeutics. ADSC based studies have mostly involved animal models, but existing human trials show their safety and feasibility as well as many improvements in a variety of conditions and diseases. Nevertheless, ADSC therapy may pose risks regarding malignant transformation, tumour progression, fibrosis, and pro-inflammation (Miana & Gonzalez, 2018)

Challenges of ADSC-based MS therapies

A caveat to bringing ADSC-based therapies to the market for the clinical treatment of MS is regulatory approval by regulatory bodies such as the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The EMA classifies cell, gene, and tissue-based therapies as "Advanced Therapy Medicinal Products (ATMPs)". ADSCs do not fall under this category as these cells are usually manipulated before administration, e.g. collagenase extraction of ADSCs from the fat tissue, meaning the cells are not homologous of the donor adipose tissue, a requirement of ATMPs (Raposio and Ciliberti, 2017). However, the advancement of modern medicine has seen the EMA approve the use of the ADSC-based treatment Darvadstrocel to treat perianal fistula in Crohn's Disease (Meng and Baumgart, 2020). The EMA has designated orphan status to other proposed ADSC-based therapies, which recognises the potential of these therapies to prevent, treat and manage orphan (i.e. rare) disorders and diseases such as epidermolysis bullosa, Buerger's disease and anal fistula (EMA, 2009, 2014, 2017; Tsigkos *et al.*, 2021). The FDA warns vulnerable patients to stay away from unproven and unapproved stem cell treatments that could be harmful (site reactions, movement of cells from placement sites, change into inappropriate cell types, failure of cells to work, and tumour growth) and that are illegal; patients that seek stem cell therapy should make sure that treatments are FDA-approved or are being studied under an Investigational New Drug Application. To date the only FDA-approved stem cell-based treatments consist of hematopoietic progenitor cells derived from cord blood (FDA, 2019).

Whilst the anti-inflammatory, immunomodulatory, neuroregenerative and neuroprotective benefit of MSCs in treating MS have been highlighted, some drawbacks have been defined. Establishing effective dosage ranges, route of administration, time between doses and the mechanism of MSC migration to the CNS will further advance and optimise MSC therapies for MS treatment (Mansoor *et al*., 2019; Barati *et al*., 2020; Hassanshahi *et al*., 2021). Controlling the fate stem cells once administered *in vivo* has proven a challenge for stem cell technology and its subsequent advancement. At present, there is no non-invasive way of observing how stem cells act *in vivo* (Tang *et al*., 2022). Consequently, several clinical trials strived to investigate MSCs as potential therapies for a variety of human diseases, like MS (Rodríguez-Fuentes *et al*., 2021).

Although ADSCs could represent a valuable treatment option for many diseases, future studies should focus on continuous monitoring and long-term follow up of ADSC transplantations, with larger controlled trials being studied. Investigations should be performed to better understand the immunomodulatory properties and network of ADSCs (Ceccarelli, *et al*., 2020). Further exploration into ADSC-derived exosomal and extracellular vesicles as they may be more effective when compared to whole cell treatments, due to the possible alteration of the immune system and pro-tumorigenic potential that ADSCs could have (Volaveric, *et al*., 2018). Optimal isolation, administration and allogeneic versus autologous ADSCs transplantations need to be standardised and regulated across studies and clinical trials. Future studies should address current pitfalls in ADSC-based therapies, with a need for further research and larger clinical trials to investigate the potential of ADSCs as novel therapeutics in the treatment of MS.

7. Conclusion

MS is a prevalent disease with the need for reliable and effective treatment options becoming evident on completion of this review. Current therapies, despite having some benefits, do not stop the progression of neurodegeneration and MS disease progression. The benefit of novel

approaches like stem cell-based therapies have been demonstrated in experimental animal models of MS and in clinical trials to date. The ability of ADSCs to differentiate into different cell types, modulate the immune system and inflammatory response is a promising characteristic that could be exploited in future therapies. These properties have been utilised in treatments for inflammatory, cardiovascular, metabolic, neurodegenerative, and autoimmune diseases, such as Alzheimer's, Diabetes mellitus, Huntington's, Parkinson's, skin diseases, Crohn's Disease and MS. Currently, the EMA has approved the use of an ADSC-based therapy for the treatment of fistula in Crohn's Disease, while the FDA has only approved the use of cord-blood derived haematopoietic progenitor cells to treat disease. Nevertheless, clinical trials have proved the feasibility and safety of ADSC-based therapies, highlighting the potential of stem cell therapies for the treatment of MS. Larger studies focusing on MS are required, not only to investigate the therapeutic benefit of such therapies, but also to predict any possible adverse effects that may occur. There is a need for current regulatory framework in Ireland to develop guidelines to regulate the use of stem cell therapies for MS treatment. The future of stem cell therapies for MS treatment may include autologous ADSC therapies and ADSCs as drug delivery systems, among others. Further studies are required to elucidate and refine a successful, safe, and effective stem cell therapy for MS treatment.

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