Journal of Stem Cell Research

Genesis-JSCR-4(2)-51
Volume 4 | Issue 2
Open Access
ISSN: 2582-8797

# The Use of Stem Cells in The Treatment of Multiple Sclerosis

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**Citation:** Geissler A, Gallicchio VS. (2023) The Use of Stem Cells in The Treatment of Multiple Sclerosis. J Stem Cell Res. 4(2):1-17.

**Received:** June 6, 2023 | **Published**: June 20, 2023



## Abstract

Autoimmune disorders are becoming increasingly common in the United States, with tens of millions of people affected. Autoimmune disorders cause a wide range of symptoms; from joint degeneration and pain to demyelination of the brain and spinal cord, these diseases have the potential to cause severe anguish and physical pain to the people affected by them. While patients with autoimmune disorder are most immediately affected, loved ones of patients also suffer emotionally, and oftentimes, financially due to the disease. One particularly debilitating autoimmune disorder, multiple sclerosis, is characterized by demyelination of the brain and spinal cord. Multiple sclerosis has detrimental effects on the central nervous system, with many patients losing the ability to ambulate or move. Multiple sclerosis has no cure, but many different drugs have been created that help patients and their families manage the effects of the disease. Unfortunately, these drugs have the potential to introduce dangerous adverse effects and infections to patients. Most drugs marketed to help manage multiple sclerosis do just that, they manage it, Most drugs marketed to help manage multiple sclerosis do just that, they manage it, Most drugs marketed to help manage multiple sclerosis do just that, they manage it, Although they do not provide a complete cure for multiple sclerosis as of now, clinical trials Although they do not provide a complete cure for multiple sclerosis as of now, evaluating the use of stem cells to treat multiple sclerosis have proven to restore some aspects of normal function, in addition to improving the overall quality of life for multiple sclerosis patients and their loved ones.

**DOI:** https://doi.org/10.52793/JSCR.2023.4(2)-51

**Keywords**
Autoimmune disorder; Multiple sclerosis; Central nervous system; Demyelination; Stem cells.

## List of Abbreviations

aHSC– Autologous Hematopoietic Stem Cells
aHSCT – Autologous Hematopoietic Stem Cell Transplantation
ATG– Anti-thymocyte Globulin
BBB – Blood-brain Barrier
CNS – Central Nervous System
CSF – Cerebrospinal Fluid
CY – Cyclophosphamide
DIS– Dissemination of Lesions in Space
DIT – Dissemination in Time
DMT – Disease-modifying Therapy
EAE– Experimental Autoimmune Encephalomyelitis
EBV – Epstein-Barr Virus
EDSS –Expanded Disability Status Scale
EFS – Event-Free Survival
ESC – Embryonic Stem Cell
G-CSF– Granulocyte Colony-stimulating Factor
GM-CSF – Granulocyte-macrophage Colony-stimulating factor
HALT-MS – High-Dose Immunosuppression & Autologous Transplantation For Multiple Sclerosis
HDIT – High-Dose Immunosuppressive Therapy
hESC–Human Embryonic Stem Cell
hiPSC – Human-Induced Pluripotent Stem Cell
HSCT –Hematopoietic Stem Cell Transplant
iPSC – Induced Pluripotent Stem Cell
JC Virus – John Cunningham Virus
MRI – Magnetic Resonance Imaging
MRSA – Methicillin-Resistant *Staphylococcus aureus*
MS – Multiple Sclerosis
MSC–Mesenchymal Stem Cell
OPC – Oligodendrocyte Progenitor Cell
PBMC – Peripheral Blood Mononuclear Cell
PBSC–Peripheral Blood Stem Cell

PFS – Progression-Free Survival
PMA–Phorbol Myristate Acetate
PML– Progressive Multifocal Leukoencephalopathy
PPMS– Primary Progressive Multiple Sclerosis
RFS – Relapse-Free Survival
RRMS– Relapsing-Remitting Multiple Sclerosis
SPMS – Secondary Progressive Multiple Sclerosis
TBI –Total Body Irradiation
UCMSC – Umbilical Cord Mesenchymal Stem Cell
VCAM-1 – Vascular Cell Adhesion Molecule-1
VLA-4 – VeryLate Antigen-4
VRE – Vancomycin-Resistant Enterococci

## Introduction

### What are autoimmune disorders?

The prevalence of autoimmune disorders is increasing worldwide. While there are a wide range of autoimmune diseases, some autoimmune disorders can be life-changing, or even worse, life-ending. There are many factors that increase one's susceptibility to developing an autoimmune disorder, such as genetic, environmental, hormonal, and immunological components. While all these factors can play a significant role, almost half of the cases of sudden development of an autoimmune disorder are attributed to "unknown trigger factors" [1]. Another important factor not previously listed, that has been proven in over 75% of cases of sudden onset, is intense physical or emotional stress leading up to the onset of symptoms. High levels of stress have also been proven to exacerbate current symptoms even more, and it has been speculated that some endocrine hormones released during stress can lead to immune dysregulation [1]. Although autoimmune disease is viewed as rare, their effect on mortality and morbidity can be drastic [2]. It is estimated that about 3% of the population of the United States has an autoimmune disorder, with the highest prevalence and mortality rates being young and middle-aged women. Autoimmune disorders disproportionately affect women, with more than 60% of autoimmune disorder diagnoses belonging to women, except for Type I diabetes and some other disorders [2]. While autoimmune diseases can occur at any age, certain autoimmune disorders have an onset within a certain age range. For example,the primary autoimmune disease that has a childhood or adolescent onset is Type I Diabetes Myelitis. The primary autoimmune diseases that occur in mid-adult years are multiple sclerosis and myasthenia gravis, and the primary autoimmune diseases that occur in older adults are rheumatoid arthritis and primary systemic vasculitis [2]. While some autoimmune diseases have no cure, the use of stem cells in treatment for some autoimmune disorders has proven effective in preventing the progression of the autoimmune disease.

## What is Multiple Sclerosis?

Multiple sclerosis (MS) can be a particularly debilitating autoimmune disorder. Roughly 2.3 million people globally suffer from MS, though this number is likely to be underreported. Women in their young adult life are most affected by MS [3]. The sex ratio was once roughly equal but has since turned increased to a 3:1 female to male ratio over the last hundred years or so [4]. It has been found that San Marino and

Germany have the highest prevalence of MS, followed by the United States. The prevalence of MS is statistically lower in low and middle-income countries than it is in high-income countries, however, this could be due to lack of medical care or inability to afford treatment [5]. While many queries regarding its susceptibility have gone unanswered, research into genetic and environmental factors have shown that low vitamin D levels, smoking and a past Epstein-Barr Virus (EBV) infection can increase an individual's susceptibility to developing MS [3].Symptomatic EBV infections has been proven to almost double an individual's susceptibility to contracting MS [4].Adult migrants that travel from low-risk areas to high-risk areas of the world maintain a low probability of getting MS. However, children born to those low-risk migrants in a high-risk area have a higher probability of getting MS. This information leads researchers to believe that while genes and environment are both factors in the onset of MS, environment may play a larger role than genetics [4]. Genetics does still play a slight role in the development of MS, as roughly 1 in 8 patients with MS have a family history of the disorder. MS is an autoimmune inflammatory disorder and is characterized by demyelination and some form of axonal loss triggered by dysregulation of the immune system [4].The pathogenesis of MS involves an immune attack against antigens in the central nervous system (CNS), which are mediated through activated $CD4$+T cells. It is probable that B cells also have a contribution to the development of MS [6]. Once the T cells are activated, they can cross the blood-brain barrier (BBB). The process of crossing the BBB occurs when the very late antigen-4 (VLA-4) that exists on T cells has an interaction with the vascular cell adhesion molecule-1 (VCAM-1) that exists on capillary endothelial cells. Once the autoreactive T cell is in the CNS, it can be reactivated during interaction with autoantigenic peptides within the brain. This reaction leads to an inflammatory response and the release of cytokines, chemokines, T cells, monocytes, B cells and macrophages, which result in myelin damage[6]. Areas of demyelination with inflammation and loss of axonal material are called lesions. These lesions primarily affect white matter of the brain, the spinal cord, and optic nerves. The inflammatory contents associated with lesions include activated T cells, activated macrophages, plasma cells, and B cells. Lesions can be classified as active, chronic or remyelinated. Active lesions are common with relapsing-remitting MS (RRMS), and usually have demyelination, but the axon is preserved. Chronic lesions are usually seen in progressive MS patients, and often display a combination of demyelination, axonal degradation, and relative absence of active inflammation. Remyelinated lesions are often seen on the edges of active lesions and contain thinly myelinated axons and high numbers of oligodendrocyte precursor cells [6]. The disease can cause permanent damage to the central nervous system and lead to the degradation of nerve fibers. Some people with severe MS may lose the ability to walk completely, while others experience periods of remission, followed by periods of symptom flare-ups [7].

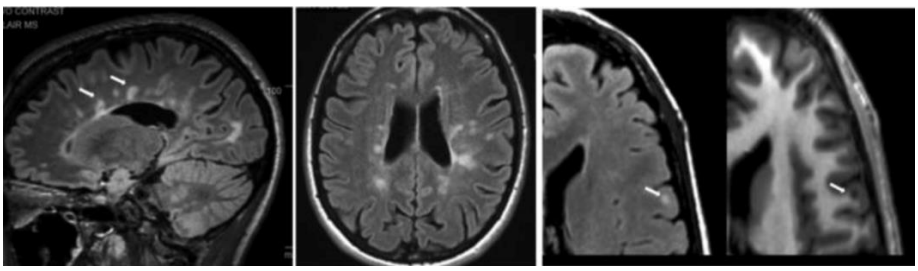

**Figure 1**: MS gray and white matter lesions in the brain shown by MRI imaging [8].

MS has two forms: relapsing or progressive. RRMS shows small episodes of neurological disability, which often leads to partial, complete or no remission from the disability, and sometimes the neurological disability that comes with it [9]. Neurological symptoms in patients with RRMS occur suddenly and usually without warning [10]. RRMS is represented in 70-80% of MS patients [11]. Patients that experience progression of the disease from the time of onset are labeled as primary progressive MS (PPMS). PPMS represents about 10% of patients with MS. Sometimes, relapses will decrease in occurrence, and a gradual, uninterrupted overall worsening will occur, called secondary progressive MS (SPMS) [9]. Diagnosis of MS can be difficult because neurological symptoms associated with the disease (tinging, facial weakness, vertigo, pain, cognitive dysfunction, sexual dysfunction, fatigue, etc.) mimic a lot of other neurological conditions. MS symptoms can also go undetected for a long time before being diagnosed. For example, symptoms of RRMS may be present for hours or days at a time and can be followed by remission for weeks or months. In addition, PPMS has slow progressive symptoms from the onset of the disease that can take months or years to catch [9]. Diagnosis is confirmed by the discovery of an inflammatory CNS injury, and the spread of disease from one CNS location to more than one location as symptoms progress. The main tests used to confirm diagnoses are magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) analysis [9]. CSF inflammatory markers are present in more than 80% of patients with MS. The most used criteria for the diagnosis of MS are the McDonald criteria. Using these criteria, the presence of neurological, MS-like symptoms at multiple points in time is studied, as well as clinical or MRI data: an approach called dissemination in time (DIT). Another important assessment to consider in diagnosis is the dissemination of lesions in space (DIS) [12]. A definitive diagnosis requires 2 or more attacks, clinical evidence of 2 or more lesions, or clinical evidence of 1 lesion with evidence of an attack in the past[6].DIS can be proven by the evidence of more than 1 lesion in 2 out of 4 locations in the CNS: periventricular, juxtacortical, infratentorial, spinal cord. While using the McDonald criteria, it is extremely important that alternative diagnoses are studied and ruled out [9].

In 2005, it was recommended that the McDonald criteria for diagnosis of PPMS require, in addition to one year of disease, 2 out of 3 of the following findings: positive brain MRI, positive spinal cord MRI, or positive CSF. The combination-like approach was recommended due to the combination of CSF and MRI effects that lead to PPMS [12]. While the McDonald criteria has been very helpful in the diagnosis of MS, it was created using data from adult Caucasian European and North American populations, so its accuracy regarding other populations, ethnicities, and age ranges is questionable. As of the 2010 revisions of the criteria, Asian and Latin American criteria have not been validated [12]. Another widely used criteria involved in MS is the expanded disability status scale (EDSS), which measures disease progression in patients. The EDSS measures the functionality of the CNS and assesses the efficacy of treatments and clinical trials in patients. The scale is measured in increments of 0.5, and ranges from 0-10, with 0 meaning there isno neurological impairment due to MS, while a score of 10 means death occurred due to MS [13].

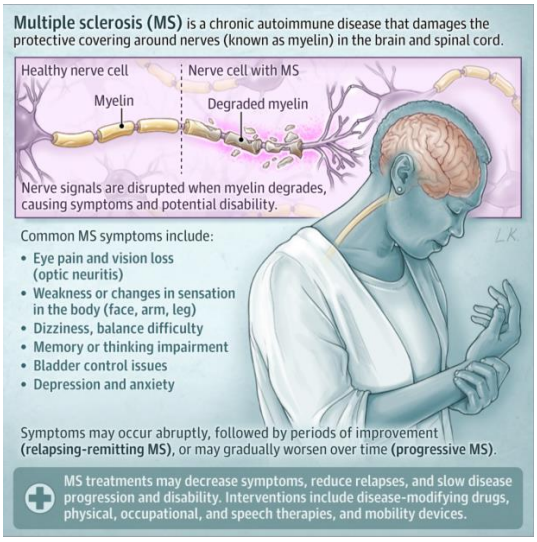


**Figure 2**: An outline of what MS is and its clinical presentation [10].

The management of MS usually includes treatment with immunomodulatory agents that help alter the course of the disease, and medications that help with symptom management [6]. Until recently, steroids were one of the only treatments for MS. There have been many new drugs created for the purpose of managing MS, but they have varied effectiveness. Since no two people are the same, determining the best course of treatment for MS patients can be difficult. The main criteria used in drug treatment efficacy in MS are based on disease activity, specifically the relapse rate and progression overtime [14]. Steroids work by inhibiting T cell activation and reducing the production of inflammatory agents. While steroids can sometimes prove effective in managing MS, they have many contraindications and adverse effects, which have made them less popular with patients and physicians; there is a need for treatments with limited adverse effects and higher effectiveness rates. Most of all, these treatments need to be safe for the patients.

## Current Drug Treatment Options

Glatiramer acetate is a synthetic amino acid polymer that resembles a basic protein, myelin, which is the protein that is broken down by T cell activation in MS patients. Glatiramer acetate promotes an anti-inflammatory immune system setting, which helps reduce the relapse rate and the number of possible active MRI lesions. Glatiramer acetate is generally safe and has minor adverse effects [14]. Interferon-β-1a and Interferon-β-1b promote the production of an anti-inflammatory immune system setting by increasing the production of anti-inflammatory cytokines and decreasing the production of inflammatory cytokines. Interferon-βreduces the movement of these cells within the CNS, which helps eliminate the formation of new MRI lesions and helps lower the overall relapse rate [14].

Natalizumab was the first monoclonal antibody approved for MS treatment. Natalizumab interacts with $\alpha 4$ receptors on the surface of T cells, which limits its ability to bind to the BBB. Since the T cells are not able to cross the BBB, they are not able to enter the CNS. Natalizumab has been proven to limit the number of new lesions and lowers the relapse rate and the probability of disease progression. While Natalizumab seemed like it may have been the sure cure, this immunosuppressant leaves patients with

an increased risk of developing progressive multifocal leukoencephalopathy (PML). Risk factors for PML include: natalizumab treatments lasting longer than 2 years in duration, prior use of immunosuppressants and the finding or anti-John Cunningham (JC) virus antibodies [14]. PML is a severe, and often fatal nervous system infection caused by the JC virus. While PML is a latent infection in most healthy individuals, it is usually fatal in patients that are immunocompromised and have one or more of the following conditions: acquired immunodeficiency syndrome, post organ or bone marrow transplant recipients, malignancies, and chronic inflammatory conditions, like MS. Additionally, each natalizumab infusion increases the likelihood of PML [15]. In patients without anti-JC virus antibodies, the risk for developing PML is roughly 0.0001% [11]. Though some drugs have been tested in their ability to treat PML, they have all been deemed ineffective; there is currently no cure for PML [15]. The possibility of developing PML has been enough for some patients and physicians to determine it should not be used in the patient's course of treatment.

Fingolimod is the first oral treatment that was available for the treatment of MS. Fingolimod engulfs the sphingosine-1 receptors that exist on T cell surfaces, which prevents the movement of T cells into blood circulation [14]. Fingolimod has been shown to reduce the loss of brain volume in MS patients in clinical trials. While Fingolimod does lower the relapse and progression rates, there are many dangerous adverse effects associated with it. For example, Fingolimod has been associated with liver dysfunction, bradycardia, leukopenia, lymphopenia, and increased risk of varicella-zoster virus infections. In this case, bradycardia occurs because sphingosine-1 receptors are also present in cardiac tissue [14]. The binding of sphingosine-1 receptors to fingolimod in cardiac tissue in animal trials have shown that there is an initial lowering of the heart rate due to the initial action of fingolimod before these receptors are engulfed [16].

While disease-modifying therapy (DMT)has a higher success rate when used in the treatment of early MS, many patients do not seek treatment due to a misunderstanding of their neurological symptoms or a fear of treatment [17]. While DMTs have shown a decrease in relapse and progression rates, there is still the need to search for a treatment that will help reverse the disease, not just manage it. Treatments are also extremely expensive because more care is needed as the disease progresses. These calls for a cure are being answered using stem cells.

## What are Stem Cells?

Stem cells are undifferentiated cells that are found throughout the body. They are present in embryonic, fetal, and adult life stages and can differentiate into different types of cells. Most stem cells share a few of the same characteristics, to a certain degree: self-renewal, clonality, and potency [18]. Some stem cells, like bone marrow, liver, lung, and gut, proliferate as necessary to supplement cells under normal conditions. Heart or nervous system cells, however, usually proliferate in response to an injury [18]. Due to their ability to self-renew and proliferate, stem cells appear to be promising in the future of regenerative medicine. Although not always classified in this manner, stem cells can be classified into different categories: pluripotent and multipotent [19]. Pluripotent stem cells can differentiate into all cell types found in the body. They are only present for a short period of time in a human embryo before they differentiate into multipotent stem cells, which form all other tissues of the body [19].

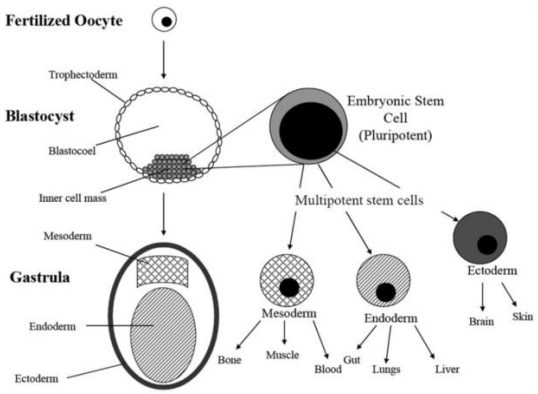


**Figure 3**: The figure shows the proliferation and differentiation of stem cells, beginning with a fertilized egg. Pluripotent stem cells are derived from the inner cell mass of the blastocyst. Pluripotent stem cells differentiate into multipotent stem cells. Multipotent stem cells can also be derived from the gastrula. Multipotent stem cells assist in the growth of various body tissues [19].

Pluripotent stem cells used in research commonly come from embryos, termed embryonic stem cells. While pluripotent stem cells show promise due to their ability to differentiate into any tissue type, multipotent stem cells also show promise. Multipotent stem cells develop into all cells in their germ layer, so there is still a range of tissues that can be regenerated using stem cells. Multipotent stem cells used in research are typically found in their parent organ and can help regenerate damaged tissue within the parent organ [19]. Multipotent stem cells taken from bone marrow, termed hematopoietic stem cells, have been used for over 50 years in the treatment of some blood cancers and heart conditions. Recently, it has been discovered that multipotent stem cells can form joints in animal trials [19]. Not only can multipotent stem cells be used in the treatments previously discussed, but they also show promise due to their immunomodulatory properties.

## Stem cell therapies for multiple sclerosis

Currently, 5 types of stem cells are being examined for efficacy in treating MS, and 3 will be discussed further: autologous hematopoietic stem cells (aHSC), mesenchymal stem cells (MSC), induced pluripotent stem cells (iPSC) [20].

## Hematopoietic stem cell therapy

HSCT was first evaluated in the 1950s using mice models, which showed that infusion of hematopoietic cells could help replace dysfunctional cells with more healthy, fully functioning cells [21]. Immunoablative therapy followed by a HSC therapy has since been studied in rats experimental autoimmune encephalomyelitis (EAE), which is a model of CNS inflammation. The idea behind this method is to eliminate harmful immune cells from the immune system, and then replace them with new immune cells using a HSC infusion. This procedure proved that a HSCs can induce remission, prevent relapse, and unlike many drug therapies, intensify the recovery of the CNS [20]. Using this technique, HSCs are moved from peripheral blood using one of two mechanisms: the use of granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), preferably with cyclophosphamide (CY). Once the HSCs are collected, they are kept in cryo conditions until the patient is ready for the transplant. CD34+ can be selected for in HSCs ex vivo, to lower the risk of putting autoreactive cells back into the

patient's body during treatment [20]. The following step, ablative conditioning, can be high-strength and uses agents like, like busulfan, CY and anti-thymocyte globulin (ATG). These regimens deplete the T cell population in the human body. Intermediate-strength ablative conditioning incorporates a combination of different chemotherapies, and low-strength ablative conditions contains a combination of CY and ATG [20].

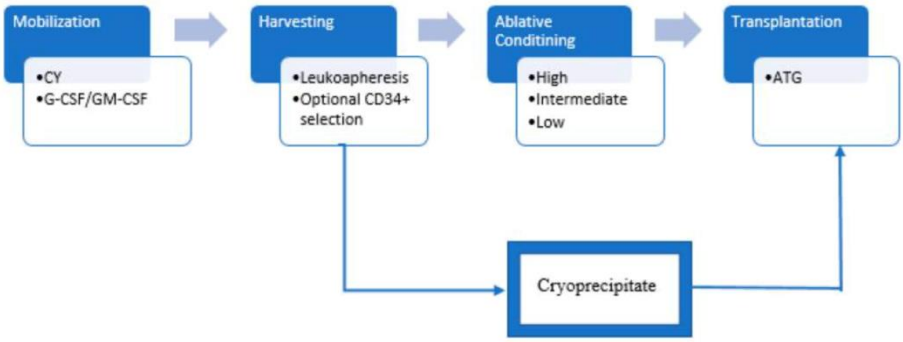


**Figure 4:** The process of mobilization of HSCs, followed by the other steps in preparation for treatment with aHSCs in patients with MS [20].

A total of 50 studies, including cohort studies, clinical trials, case-control studies, and case series, containing 4,831 patients with MS were included in a comprehensive review on the use of aHSCs in the treatment of MS [22]. A select few clinical trials will be discussed below [23] harvested immune cells using CyTOF protocol, to help determine if autologous stem cell therapy and high-dose immunosuppressive therapy are beneficial to patient treatment. High-dose immunosuppressive therapy (HDIT), followed by hematopoietic stem cell transplant (HSCT)has become common for patients with RRMS. Peripheral blood mononuclear cells (PBMC) were taken from each patient and were cryopreserved, as well as shipped in liquid nitrogen to preserve the cultures. Some PBMCs were thawed and mixed with 10 ng/mL phorbol myristate acetate (PMA) and ionomycin, while others were left untouched. Both groups of cells were kept at 37C for 4 hours, while being exposed to brefeldin and monesin [23]. Cells were stained using a combination of compounds, under a protocol called CyTOF. CyTOF allows for data to be collected from cytokines and chemokines, in addition to certain surface cell antigens and T-cell components, the primary cell type affected by MS [24]. Under the CyTOF protocol, cells are first activated by a drug cocktail and a protein transport inhibitor is commonly added to ensure cytokines remain within the cell, followed by the removal of certain cells from activation. Antibodies to certain cell surface markers are added to the cells, then the cells are fixed [24]. During fixation, the cells are also permeabilized, which is usually accomplished using certain agents, like saponin, methanol or acetone. Permeabilization allows for access to intracellular/intraorganellar antigens within cells [25]. Once the cells were permeabilized and stained, mass cytometry was used for analysis [24].

[26] later used a similar harvesting technique to determine the efficacy of HSCT/HDIT treatment in 24 RRMS patients in the high-dose immunosuppression and autologous transplantation for multiple sclerosis (HALT-MS) trial[26].The goal of this clinical trial was to determine if this course of treatment has the potential for event-free survival (EFS), meaning patients survive and have no disease activity, which includes, but is not limited to increasing disability level, relapse, or new MRI lesions. The HALT-MS trial

was trying to test the validity of the idea that if one of the body's immune system cell populations is depleted and newer, healthier cells are introduced, patients with MS may be able to reach EFS. Patients were between the age of 18-60 years and were diagnosed with MS using the McDonald criteria. All patients had RRMS, and EDSS between 3-5.5, lesions on a brain MRI that are found in MS patients, a disease duration of less than 15 years, and a failure of DMT. In this trial, failure of DMT is defined as more than 2 relapses over the course of 18 months and an EDSS increase [26]. Peripheral blood stem cells (PBSC) were administered via infusion on day 0. The HDIT in this clinical trial included fractions of total body irradiation (TBI). Two TBI treatments per day, for 2 days were given to patients, and these treatments were conducted at least 5 hours apart. In addition to TBI and other intravenous medications, prednisone was given to the last 8 patientsfrom days 7-21 to prevent complications in the CNS during engrafunent, due to the fact the first 18 patients of the study suffered from engrafunent syndrome, which led to neurological symptoms in some patients [27]. The 24 patients that underwent the HALT-MS trial had a median age of 37, a median disease duration of 4.9 years, and had a baseline EDSS of 4.5 [26]. Two participants in the trial had disease progression and ultimately died. Another patient suffered from disease progression at 15 months and died 4.5 years post-HSCT. The cause of death in all 3 patients was cardiopulmonary arrest. After 4 years, the probability of EFS was 73.8% and After 69.2% after 5 years. 7 patients did not maintain EFS, and either had an increase in EDSS greater than 0.5, new MRI lesions, or relapse. Overall, the 5-year progression-free survival (PFS) was 91.3%. In addition, 86.9% of patients had not had a relapse and 86.3% of patients were MRI activity-free. Overall study survival was 86.3% [26]. While there are still slight adverse effects due to this treatment, it shows a promising step towards a new MS treatment.

[28], conducted a cohort study in people with MS to determine how effective autologous hematopoietic stem cell transplantation (AHSCT) is in real patients. Data was collected on 120 patients who received AHSCT treatment for MS between February of 2012 and January of 2019 out of Kings College Hospital and from April 2016 to January 2019 at Hammersmith Hospital in London, UK. Since there were more than 120 patients total who had received this treatment, inclusion criteria were created to select patients [28].

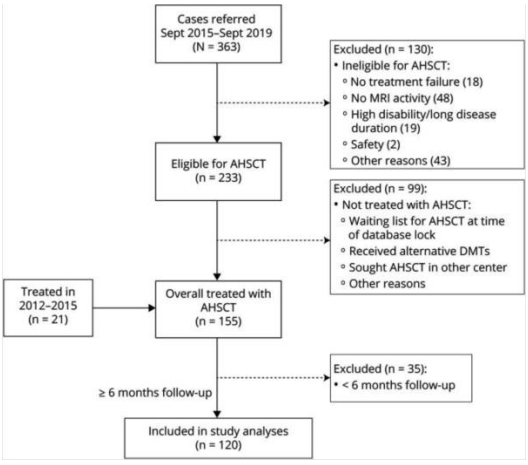


**Figure 5**: Treatment inclusion criteria used by Nicholas et. al during the study of the efficacy of AHSC transplantation in two London, UK hospitals [28].

62 patients in the London trial had progressive MS and 58 patients had RRMS. The median EDSS score out of the 120 total patients was 6. At King's College Hospital, PBSCs were mobilized after CYand a 7-day administration of G-CSF occurred until leukapheresis. At Hammersmith Hospital, PBSCs were mobilized with CY and daily G-CSF was administered until leukapheresis. After transplantation occurred, supportive medical treatments were provided to patients as needed [27]. EDSS scores were evaluated for 12 months prior to treatment and up to 12 months after treatment in all patients. An increase in EDSS in this trial was defined as an increase >0.5 in patients with a baseline EDSS>6, and an increase of >1.0 in patients with an EDSS<6. Patients underwent their first MRI scans roughly 6 months after AHSCT. 93% of patients in the trial were relapse-free after 2 years, and 87% were relapse-free after 4 years. In some patients with RRMS, relapse did occur, however, the relapse-free survival (RFS) in RRMS patients overall was 77% after 4 years [28]. The average EDSS score change was roughly 0.25 during the year leading up to AHSCT. In the year following AHSCT, the average EDSS change was 0.02. Although the results of AHSCT are promising, it is important to note that 90% of patients in this trial suffered from at least 1 early adverse effect after transplantation. Fever and neutropenia rates were higher in the Kings College Hospital patients after transplantation, possibly due to a higher dose of CY used in mobilization. The Kings Cross Hospital patients experienced more fever, diarrhea and EBV reactivation, while the Hammersmith Hospital patients experienced more nausea and vomiting [28]. Three patients involved in the study died within 100 days of transplantation; 2 of these patients had PPMS and 1 had RRMS. At the beginning of the study, all 3 patients who died had a baseline EDSS of 6.5, indicating strong neurological impairment due to MS. 2 deaths occurred before AHSCT, and in the third patient, death occurred roughly 30 days after AHSCT due to sepsis and respiratory distress [28]. In progressive forms of MS, AHSCT treatment has shown it does not reduce progression in some patients with SPMS [29]. Although some adverse effects occur with AHSCT treatment, this treatment has shown to be a promising future treatment in patients with more aggressive forms of MS.

[30], conducted a clinical review of the use of non-myeloablative AHSCT in 507 patients with RRMS and SPMS that received the treatment at Northwestern University between July 2003 and October 2019.All patients needed to be free of DMTs for a certain amount of time before they were able to participate in AHSCT, to reduce the possibility of PML [30]. PBSCs were collected in an outpatient facility, and patients also received CY, followed by filgrastim 5 days after. On admission to the study, tests for methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) were performed on patients. The patients had a median age of roughly 37, and most participants were female. 376 patients were treated with CY and ATG; 28 patients were treated with CY, ATG and rituximab (500mg); 35 patients were treated with CY, ATG and rituximab (1000 mg); 46 patients were treated with CY, ATG and intravenous immunoglobulins; 26 patients were treated with CY and alemtuzumab [30]. One death occurred post-transplantation due to hospital-acquired legionella pneumonia. 1.1% of patients suffered from Clostridium difficile, and 1 patient had bacteremia. For the entire cohort, 98.9% of patients had RFS. Out of the 414 patients with RRMS, 33 patients relapsed. PFS occurred in 475 patients, and PFS for the entire cohort after 4 years was 95.0037%. The patients with RRMS had an EDSS that improved by roughly 1.5 after 5 years. For patients with SPMS, the EDSS improved slightly during the first-year post-transplantation but did not improve any further [30]. While non-myeloablative AHSCT may not be the

most effective treatment in patients with SPMS, treatment by non-myeloablative AHSCT shows a drastic improvement in EDSS in RRMS.

[31], evaluated AHSCT in aggressive types of MS that do not respond to conventional DMTs. To be eligible, patients had to receive AHSCT for MS between 1995-2006, a baseline EDSS, information on the administered regiment and follow-up availability. Data was collected from 25 treatment centers in 13 different countries for 281 patients. The median follow-up was 6.6 years, and 186/281 of patients had SPMS [31], with a median EDSS of 6.5. PFS was found in 45% of patients after 5 years post-AHSCT, and most patients with SPMS did not have an increase in EDSS score after 5 years. The overall survival rate of the study was 93% after 5 years (37 patients died from various causes during the 6-year follow-up period). Most patients with positive results were younger and had lower EDSS values. Patients with a baseline EDSS >7 had a higher chance of mortality. This large cohort demonstrates that when MS is treated early using this technique, patients may have a higher chance of PFS [31].

## Mesenchymal stem cell therapy

MSCs are non-hematopoietic adult stem cells that can self-renew. These stem cells originate from the mesoderm but can differentiate towards endoderm and ectoderm cells as well [32]. MSCs also have similar properties to pericytes, and they are beginning to come more popular in the possible treatment of certain disorders [33]. MSCs can be found in almost all tissues in the human body but were first found in bone marrow. Evidence has shown that MSCs can assist in the regrowth of bone, fat, and cartilage, and in some instances, they have the potential to become muscle cells or neurons under certain conditions [33]. In experimental models, bone marrow MSCs administered through the intraperitoneal route showed a decrease in inflammatory response and demyelination in the spinal cord. Bone marrow MSCs also showed to inhibit CD4+ and CD8+ activation [32]. Umbilical cord mesenchymal stem cells (UCMSC)have a wide range of culture methods and have been proven to differentiate faster than other stem cell types. Cell collection is typically painless and easy, so UCMSCs have become an attractive option in the possible treatment of MS [34]. Although MSCs have become popular in the treatment of some disorders, it is important to note that MSCs can become immunosuppressive when under the influence of certain cytokines, which would not be beneficial to patients with MS [35]. However, treatment with MSCs shows a lot of promise for future MS treatments.

[36], tested 20 subjects by treating them using intravenous infusions of UCMSCs. The UCMSCs were isolated from tissue obtained from full-term, healthy births that consenting mothers donated to the study, and these UCMSCs were produced by MediStem Panama Inc [36]. Cells were assessed between passages to ensure the health of the cells and to ensure contamination did not occur. Before treatment began, the MS diagnosis was reconfirmed in these patients using the McDonald criteria after enrolling. Patients received a specified dose of UCMSC intravenously over 7 visits. The mean age of patients in this study was 41, and 60% of patients were female. 15 subjects had RRMS, 4 had PPMS, 1 had SPMS. All subjects survived the study with no serious adverse effects [36]. A common adverse effect was headache; however, headache is common in patients with MS after receiving an MSC transplantation [37]. During the 1-year post-treatment MRI scans, 83% of patients had no new lesions or disease progression. All patients saw improvement in sexual dysfunction, walking and improved quality of life, among other things [36].

[38], evaluated the effect of USMSCs on monkeys using the EAE model, which is the most commonly model to mimic MS. To evaluate the therapeutic effect of USMSCs on MS, researchers intravenously transplanted UCMSCs into cynomolgus monkeys with EAE. The results showed that UCMSC transplantation significantly improved the clinical symptoms of MS. MRI and clinical signs indicated that demyelination was somewhat decreased after UCMSC therapy. In addition, the present study showed that the immunomodulatory functions of USMSCs had a positive effect on T-cell lineages in this EAE model[38].

## Induced pluripotent stem cell therapy

iPSCs are like embryonic stem cells (ESC), in that they can both differentiate into cell types that represent all three germ layers. iPSCs are grown in vitro and are taken from a patient's fibroblast. After these iPSCs differentiate, they can be transplanted back into the patient; the goal is to use iPSC derivatives [39]. iPSCs have been able to overcome some of the ethical concerns associated with certain MSC treatments where human embryos are used, and it has the potential to overcome immune rejection that occurs in other transplantation procedures [40]. In addition, it has been shown that patient-derived iPSCs have great potential in treating multiple autoimmune disorders, due to the ability to study disease-affected cells, which would allow for a greater understanding of certain disease mechanisms [41].

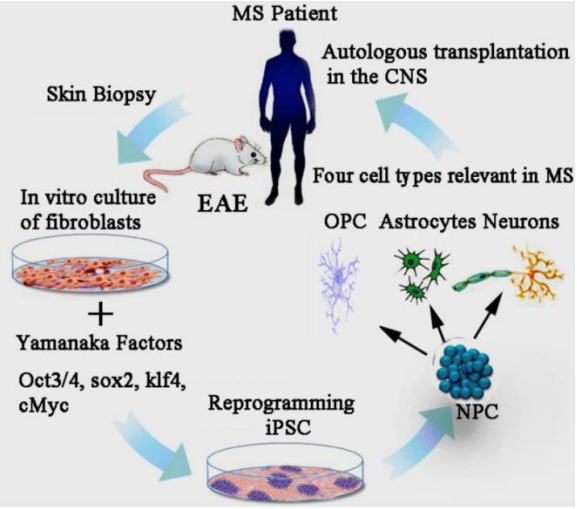


**Figure 6:** the idea behind iPSC-based treatments in humans with MS [41].

[41], differentiated human induced pluripotent stem cells (hiPSC) into oligodendrocyte progenitor cells (OPC). Using hiPSCs and hESCs, human OPCs were developed. iPSCs were used to create populations of astrocytes and oligodendrocytes, which are damaged in patients with MS. This technique also showed high levels of remyelination in mice, which could possibly have some clinical application in humans soon [42]. This technique showed to increase the survival of mice that were deficient in myelin, which suggests the use of hiPSC-derived OPCs in the treatment of demyelination [43].While many clinical trials using iPSCs in the treatment of MS have not been conducted yet, iPSCs show a lot of potential in being used to treat MS in the future.

### The price of different MS therapies

The increase in treatment cost over time in MS is typically a result of increasing disability level, along with intermittent relapses that require more care or treatment [44].The high cost of treatment is partially due to the extensive use of the healthcare system and a decrease in the ability to work in patients with MS [45]. Patients in the U.S. using DMTs, have an average annual cost of $8,500 - $54,000. MS is the second most expensive condition, second only to congestive heart failure [44]. First-generation DMTs, which were generally cheaper for a long period of time, now cost $60,000 on average annually. For example, the cost of Interferon-ß-1b, which is the oldest DMT available for MS patients, is now over $61,000, which is more than 6 times its original cost [46]. Stem cell therapy is still very new but can generally be found in most places. In the U.S. stem cell therapy costs roughly $7,000 - $10,000 per treatment (not specific to the price for MS), with treatments outside of the U.S. being higher [44]. While stem cell therapy is costly, the hope is that patients will only need a few treatments, and then will not have to pay for any treatment in the future, unlike other therapies, which are usually used for the duration of the patient's life.

Currently, the only FDA-approved stem cell transplantation treatment is HSCT, used in the treatment of blood cancers and some immune disorders, but not currently for MS, even though it is performed in clinical trials [47]. HSCT is very highly specialized and often requires a specialized care regimen before transplantation can occur, followed by a high level of care after. HSCT cost has been reported as being between $87,000 - $300,000. After the first 100 days, the median healthcare cost for myeloablative HSCT was roughly $289,000, while nonmyeloablative treatment had a median healthcare cost of roughly $253,000 after 100 days [48]. From 2004-2007, HSCT was one of the most popular procedures that had the highest increase in hospital costs, rising from $694 million to $1.3 billion [49]. However, the overall increase in cost can partially be attributed to the increase in patients who have undergone HSCT. For example, there has been a 196% increase in the number of patients who have received HSCT [50]. While HSCT treatment is very expensive, its effectiveness in clinical trials show that patients with MS may only need one HSCT to have RFS, PFS and an increased quality of life. Since drugs represent up to 82% of all costs in patients with MS, it could be beneficial to front-load the cost of treatment, rather than paying annually for medication, and paying more for supportive care as the disease progresses [45]. AT this point, the FDA has called for further study into the risk and efficacy of stem cells in treating multiple sclerosis [51]. The hope is that as clinical trials begin to show better quality of life and reversal of some aspects of MS disability, stem cell treatment for MS will be approved.

## Conclusions

Stem cells have great potential to be the primary resource for the future treatment of MS. Since the use of stem cells to treat MS is still a new approach, there has not been substantial research and clinical testing in their application for MS. The occurrence of more animal and human clinical trials for iPSCs and UCMSCs is likely to occur soon since some are already in progress. Once more information is learned about how stem cells can be used to treat MS, researchers will be able to modify treatment approaches and give MS patients a better chance at stopping disease progression and increasing patient quality of life. Learning about MS and its pathology will also help researchers modify treatment approaches using stem cells. Presently, HSCT is the most effective stem cell treatment for MS, and even though it is expensive, it can give patients an improvement in their clinical symptoms and quality of life much more than other drugs

have. As more is learned about MS and stem cell therapy for MS, stem cells have the potential to be one of the most effective treatments on the market for MS.

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