From Promise to Practice: CRISPR-Based Therapies for Multiple Sclerosis

Sze kay Tan

Abstract

More than 2.3 million people have been diagnosed with multiple sclerosis (MS) worldwide (Doshi and Chataway). MS is an autoimmune condition caused by immune cells attacking the body's own central nervous system (CNS). Currently, no treatments exist, and strategies are aimed at slowing the progression and symptoms of the disease. However, novel research has cast light on certain immune cells and genes responsible for the development and progression of MS. Given this, gene editing is a promising method to treat MS outright by editing the genes of cells implicated in MS. There has been admirable clinical success with editing genes in other diseases, like sickle cell anemia, which seeds hope for curing MS using gene editing. However, there are still major challenges to consider with editing the CNS. While there are new strategies in development, many safety and ethical factors must still be addressed before this technology can be used to treat patients. Overall, this paper presents a systematic review of the potential of CRISPR as a treatment method for MS. Some biological challenges include the presence of the blood-brain barrier (BBB), immune cell heterogeneity, and off-target effects, while technical challenges include the packaging and transportation efficiency to carry CRISPR. Parallelly, novel approaches to tackle these challenges will also be addressed, such that MS may be treated, rather than just managed.

Keywords: Multiple Sclerosis, autoimmune disease, CRISPR, Central nervous system, Microglial cells, Adeno-associated viruses (AAV)

Introduction

Multiple Sclerosis (MS) is an autoimmune disease that targets the body's central nervous system (CNS). MS is a global problem, and its prevalence is rising (Murray). This neurological disease affects approximately 2.3 million people worldwide and is most prevalent in North America, with 140 cases per 100,000 people (Doshi and Chataway). The disease usually occurs between the ages of 20 and 50 years, and is twice as common in women than in men (Arneth). This chronic disease happens when the body's immune system attacks myelin in the brain. Myelin is an insulating sheath that is formed around the nerves, especially the nerves in the brain and spinal cord. This insulation allows electrical impulses to transfer quickly and efficiently along nerve cells. MS results in weakening of the BBB and demyelination in the brain, which can disrupt signals between the brain and the body, leading to nerve cell damage (Figure 1). Many symptoms of MS severely disrupt the daily lives of patients. When the cerebrum, brain stem,

visual pathway, spinal cord, and cerebellum are affected, the patient experiences disruptions in sensory, visual, and autonomic systems (Figure 2) (Blauth et al.). Overall, the more the disease progresses, the more accumulation of disability and relapses.

Different immune cells are implicated in MS pathogenesis, including T-cells, B-cells, and microglia cells. One major type of immune cell involved in the development of MS is the autoreactive T-cell. CD4+ T-cells act by infiltrating the CNS and inducing inflammation (Kendirli et al.). Another immune cell intensely involved in MS is the B-cell. Research by Fassi *et al.,* indicates that B-cells affect MS development by targeting auto-antigens. Auto-antigens are harmful as they trigger an immune response in autoimmune diseases, MS (Fassi et al.). Lastly, microglial cells are strongly implicated in MS development and similarly target myelin cells, resulting in demyelination. When there is microglial inflammatory activity, the buildup causes demyelination, which in the end leads to nerve damage (Yong). Hence, there are many different immune cells involved in the development and progression of MS. MS does not have a cure; however, there are a few treatment options. The current treatment methods for MS are antibody-mediated therapy, symptomatic therapy, plasma exchange, and pharmacotherapy. However, there are limitations to these treatments as they primarily slow the progression of the disease, but do not stop it. Therefore, new treatment methods are critically needed to treat this disease and improve the lives of people suffering from MS. In this paper, the challenges and strategies for using CRISPR technology to treat immune cells for MS disease will be thoroughly discussed.

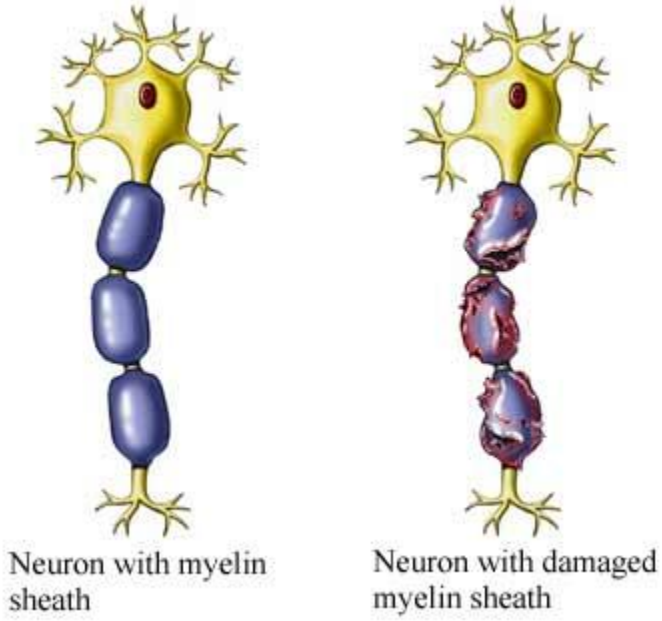

Figure 1: Damage to the myelin sheath in the autoimmune disease, Multiple Sclerosis. Diagram sourced from: [Multiple Sclerosis | Upstate Neurological Institute]

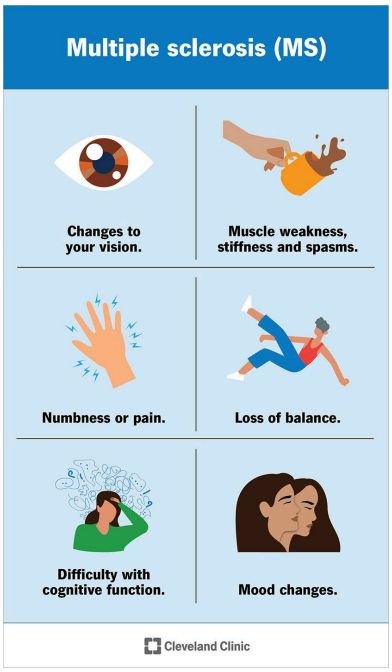

Figure 2: Most common symptoms for Multiple Sclerosis
Figure sourced from: Multiple Sclerosis (MS): What It Is, Symptoms & Treatment

CRISPR as a potential tool for treating MS

Clustered regularly interspaced short palindromic repeats (CRISPR) has shown great potential as a therapeutic tool in immune modulation since it was first discovered in 1987 by Ishino *et al.* CRISPR is a revolutionary tool for genome editing; it can be used as "molecular scissors" that precisely cuts and modifies DNA sequences (Ishino et al.). This is a remarkably hopeful tool that could help reduce the commonality of hereditary diseases. Using CRISPR technology, one can edit immune cells in the CNS that would otherwise cause an autoimmune attack or demyelination (Figure 2). There are two methods for gene editing using CRISPR: *ex vivo* and *in vivo* approaches. *Ex vivo* genome editing involves cells being removed from a body, being treated, and then being returned. There has been *ex vivo* CRISPR success for modifying immune cells, and several have reached clinical trials. *In vivo*, CRISPR involves transporting edited genes directly to the body, using delivery methods such as intravenous injection, an IV or through local delivery to a specific organ. However, *in vivo* approaches still encounter challenges due to the need to improve safety, control of viral dosage units, and efficiency (Alsaiari et al.).

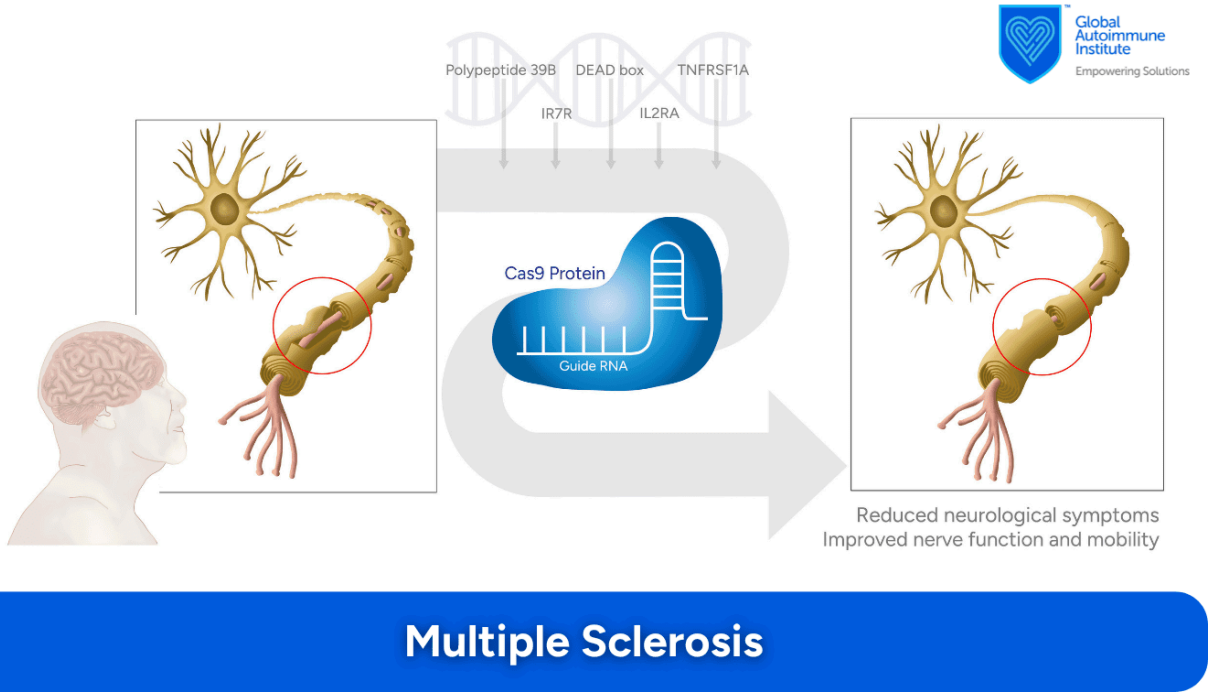

Figure 2: With the help of CRISPR, genes in the immune cells responsible for attacking the myelin sheath are edited to reduce the neurological symptoms and prolong the destruction of the myelin sheath.
Diagram sourced from: [CRISPR/Cas9 Gene Editing: Revolutionizing Autoimmune Disease Treatment]

Delivery of CRISPR components to CNS: Challenges and strategies

For optimal use of CRISPR, delivery of large macromolecules (gRNA and Cas9) into the targeted cells is necessary, along with the delivery of other macromolecules to ensure consistency of editing.To ensure the efficient delivery of CRISPR to its target cells, it is packaged in viral or nonviral vectors. This is necessary because it allows for the CRISPR components to cross the cell membrane, and gain access to the DNA for editing. One example of a viral vector are adeno-associated viruses (AAV), a small, non-pathogenic virus, that can effectively package CRISPR components.

Nonviral vectors include lipid nanoparticles and polymer-based nanoparticles (Kim et al.). However, a central technical challenge remains in how to deliver CRISPR tools effectively to immune cells within the CNS. Since MS is a CNS disease, there are many biological and technical barriers. Biological barriers include the BBB that limits CRISPR from reaching the brain, immune cell heterogeneity, and off-target effects. Some technical barriers include the packaging size of viral vectors and the immune responses they elicit. Therefore, this paper will

investigate the challenges (Table 1) and strategies (Tables 2 and 3) for delivering CRISPR tools to immune cells in the CNS involved in MS.

Challenges in delivering CRISPR components to MS-associated immune cells in the CNS

Biological barriers

[1] Blood Brain Barrier (BBB):

Overcoming the biological and technical hurdles of delivering CRISPR to MS-associated immune cells remains one of the field's greatest challenges. First, a major obstacle is the presence of the BBB. The BBB can be thought of as an airport security system, where passengers (molecules) line up to enter, but they can only get through if they meet strict screen standards. Some get through by carrying boarding passes (transport proteins), while others are denied entry. Specifically, the BBB is made up of endothelial cells lining the inside of the brain's blood vessels. This is a semipermeable and selective system whose function is to separate the blood from the brain's extracellular fluid. Primarily, it will protect the brain from foreign substances in the blood (Wu). Since CRISPR components need to be delivered to the brain to edit the genes of central nervous system immune cells, the BBB is a biological barrier that can prevent these components from reaching their target (Kumar et al.). The BBB excludes more than 98% of small-molecule drugs and all macromolecular therapeutics from access to the brain (Pandit et al.). Some developments have been made to predict the permeability of the BBB, such as clinical trials or computational methods using machine-learning algorithms (Kumar et al.). However, most drugs that infiltrate the membrane are still unable to accumulate in the brain (Malkani). Various strategies for permeability regulation are under development. For example, an intrathecal drug is a drug delivery method that can enter the ventricular system without passing through the BBB (Shah). Another method is convection-enhanced delivery, a technique that directly infuses medications into the brain (Barua et al.). However, overall, the BBB, just like airport security, has a highly selective system. Therefore, it remains a significant obstacle to deliver CRISPR to the CNS.

[2] Defense mechanism of CNS:

The CNS is commonly known with "immune privilege", a term that recognises CNS having its own specialised immune system that can limit inflammation. Firstly, the key reason for this "immune privilege" is because of the BBB which limits entry of harmful substances. However, even after this BBB has been passed, there is a strong immune system within the CNS itself. Some mechanisms that give the CNS immune system an advantage include the BBB, its limited lymphatic drainage, absence of antigen-presenting cells and presence of anti-inflammatory factors (Proulx and Engelhardt). In terms of immune cells, there are two key components of the

CNS immune response: resident immune cells and infiltrating immune cells (Ransohoff and Brown). Resident immune cells include microglia that can detect pathogens and initiate immune response, Astrocytes that contribute to immune signaling, and border-associated macrophages (CAMs) which support surveillance of the CNS. Therefore, when the CRISPR components are packaged into viral vectors and injected directly into the target site, a strong defense system may still trigger the immune response and fight against it.

[3] Immune cell heterogeneity:

MS is popularly known as the “disease of 1000 faces,” primarily due to the immune cell heterogeneity that poses another significant obstacle in its treatment. This heterogeneity is evident not only in the various gene mutations involved, but also in the diverse immune cell types that contribute to the overall disease process. There are many different immune cell key players in MS: T-cells, B-cells, microglia cells, lymphoid cells, and myeloid cells. Additionally, MS is a disease that shows a multitude of symptoms that occur unpredictably; there are many layers of impairment that individuals can be affected with. Among these are symptoms like visual disturbance, cognitive symptoms, motor impairment, bladder and sexual dysfunction, and fatigue (Engelhardt et al.). But each patient’s MS is different; one patient may only have visual impairment, while another could have motor impairment and cognitive symptoms. CRISPR treatments designed for one patient may not work for another patient affected with the same disease (Engelhardt et al.). This is complicated because there is heterogeneity for mutations and cell types. Overall, the heterogeneity of MS makes it difficult to design a one-size-fits-all treatment for patients. The need to custom-design each treatment for each patient will not only be time-consuming but also costly. This will slow down the progress of CRISPR therapy for MS patients.

[4] Off-target effects:

Another big concern is the off-target effects of CRISPR. This occurs when DNA is erroneously edited off target, leading to unintended mutations, including deletions, insertions, inversions, and translocations. Some off-target effects could also activate cancer-causing genes and trigger immune responses (Guo et al.). Off-target effects are also hard to detect because they can happen in non-coding regions of the genome and lead to unseen consequences (Hunt et al.). Because there are heterogeneous mutations involved in MS, it can be challenging to develop particular sequences(Hunt et al.). This can lead to an increased risk for off-target edits. Even when the risk of off-target effects are known, the side effects or implications may be hard to detect because the CNS is edited directly. Therefore, large ethical debates are occurring regarding whether the CNS should be edited by CRISPR at all (Salomonsson and Clelland). Methods for assessing the off-target effects of CRISPR have evolved, yet balancing the accuracy and sensitivity is still a limitation (Guo et al.).

Technical barriers

[1] Transportation:

Technical barriers are also a problem, specifically the transporting of CRISPR to the associated immune cells. Firstly, the transportation of CRISPR could potentially be encapsulated within viral vectors, a harmless form of a virus used to deliver genetic material. Specifically, an adeno-associated virus (AAV) is a popular viral vector. AAV has already demonstrated promising results in trials for CNS diseases, including neurodegenerative diseases, neuromuscular diseases, and lysosomal storage (L. Kang et al.). AAV is a non-enveloped protein capsid that can be used to transport CRISPR to target cells for gene editing. Although AAV gene therapy has proven effective in most CNS clinical trials, the limitations that were observed showed many side effects (Wang et al.). Firstly, AAV vectors are still primarily a virus and have a risk for unwanted immune responses; many factors can increase the risk of immunogenic responses (where the body's immune system fights the virus). AAV also has a small packaging capacity, restricted to 5kb. This limits the ability to transfer larger disease genes. An optimal AAV vector must have maximum yield in production, low pre-immunity (when someone has pre-existing immunity to this virus), and precise targeting.

[2] Gene editing efficiency:

Secondly, gene editing efficiency, especially for non-dividing cells, is an issue primarily linked to difficulties of delivery to the brain. The main method for gene editing using CRISPR relies on the cell's own repair mechanisms to make the desired edit, these mechanisms are linked to the cell's cycle of growth and division (Meneghini et al.). However, in post-mitotic, non-dividing cells, there is no duplication of DNA or division. Therefore the key proteins needed for gene editing are low or inactive (X. Kang et al.). This is important because non-dividing cells specifically include microglia cells in the CNS. Microglia cells are vital for myelination and have an innate immune response, therefore important for MS treatment. However, since they are non-dividing, this leads to inefficient gene editing by CRISPR and increases of unpredictable and disruptive mutations at the target site.

[3] Epigenetic restrictions:

Epigenetic restrictions include the heterochromatin, this is a tightly packed chromatin which makes up a condensed structure made up of DNA, RNA, and proteins that together form the chromosomes in the nucleus (NIH, 'Definition of Chromatin - NCI Dictionary of Cancer Terms - NCI'). Heterochromatin typically forms "condensed" foci which is localized to either the nuclear

or nucleolar periphery (Peterson). Heterochromatin is densely packed and inaccessible, which physically prevents transcription factors and other proteins from binding to the DNA. This then makes the genes inactive and less accessible to the CRISPR machinery to penetrate. Therefore making it difficult for the enzyme to bind and cut DNA for genetic modification. However, this heterochromatin can be unwound through processes that modify the chromatin structure. CRISPR systems can be fused with epigenetic effector domains like histone modifiers to reprogram a target site. Instead of the nuclease active Cas9, catalytically dead Cas9 (dCas9) can be used to bind to DNA without cleaving it. When the dCas9 is fused to an effector, it can then open up the heterochromatin to a euchromatin and promote gene expression (Kallimasioti-Pazi et al.).

Table 1: Challenges to deliver CRISPR components into the CNS

CRISPR delivery challenges		Expected outcome	References
Biological barriers	BBB	Delivery of CRISPR components to the brain is highly regularized	(Wu) (Kumar et al.) (Malkani)
	Strong defense mechanism by CNS	Immune response is mounted against the viral vectors packed with CRISPR components	(Proulx and Engelhardt) (Ransohoff and Brown)
	Immune cell heterogeneity	Diverse immune cell types contribute to the disease.	(Engelhardt et al.)
	Off-target edits	Unintended mutations introduced leading to unpredictable consequences	(Hunt et al.) (Guo et al.)
Technical barriers	Transportation of CRISPR components	An ideal vector is with a smaller size, (non-enveloped virus), good packaging capacity, low pre-immunity and precise targeting	(L. Kang et al.) (Wang et al.)

	Gene editing efficiency	Slowly dividing, limited cargo capacity of glial cells	(X. Kang et al.) (Meneghini et al.)
	Epigenetic restrictions-heterochromatin	Tightly wound DNA is inaccessible to CRISPR enzymes	(Peterson) (Kallimasioti-Pazi et al.)

Strategies for effective CRISPR delivery to the CNS

To combat these biological and technical barriers required to edit immune cells in the CNS implicated in MS, many strategies are in development. Firstly, viral vectors that contain CRISPR editing components can be modified for improved CNS delivery. Some delivery vehicles include: physical methods like microinjection, viral methods using AAVs, immune cell modification, and non-viral methods and nanoparticles (L. Kang et al.). Viral vectors are the most common delivery method currently, as they are non-immunogenic, easy to manipulate in the lab, and are relatively efficient at transferring the CRISPR components to target cells. However, there are limitations to AAVs, and therefore, non-viral vector systems, such as lipid nanoparticles and microinjection, have emerged to overcome some of the limitations of AAVs, particularly in crossing the BBB. Various strategies for overcoming the barriers to CRISPR component delivery are tabulated in Table 2.

Viral vector approaches

[1] Requirements for a successful viral vector:

Viral vectors have potential for CRISPR delivery to the CNS due to their ability to transport without causing any inflammation or toxicity to the body (Gonçalves). An ideal vector should have high-transduction efficiency, meaning it is efficient at delivering CRISPR components to target cells. It should also have an acceptable safety profile, and a large enough carrying capacity to deliver CRISPR components (Asmamaw Mengstie). Firstly, due to the highly selective BBB, a vector must be small enough (approximately ≤60nm) to make it across the BBB to deliver the CRISPR components for editing. Additionally, the vector needs to avoid blood accumulation in the liver, while also ensuring that it does not degrade during its journey to the CNS (Salomonsson and Clelland). Overall, there have been over 100 different AAV variants identified. Indeed, the expanded library of CRISPR viral vectors expands the options available to scientists to deliver AAV CRISPR components to target cells (Gray et al.).

[2] Delivery of CRISPR ribonucleoproteins:

An example is the delivery of CRISPR ribonucleoproteins (RNPs), a molecular structure that regulates RNA processing and speeds up enzymic activity (Ule). RNPs are pre-assembled complexes of the Cas enzyme and guide RNA that are delivered directly into cells for immediate gene editing. There are many benefits to using CRISPR RNPs, they can bypass transcription and translation within the cell which can allow faster editing. Most notably, RNPs can also reduce off-target and immunogenic effects. A study by Chen *et al.* showed that the self-delivery capability allowed a high level of genome editing in neural progenitor cells (multipotent neural stem cells) with low dosages of RNPs still showing minimal cytotoxicity compared to other delivery methods. This shows that RNP-based genome editing of the genes in the brain could have a potential use for self-delivery to other cell types (Chen et al.). Overall, viral vectors can transport CRISPR well, but will need to overcome a few barriers.

Non-viral vector approaches

[1] Lipid nanoparticles:

Non-viral approaches, although not as widely used, can also be used for transportation of CRISPR components instead of viral vectors. Some examples of non-viral approaches include lipid nanoparticles, nanoparticles, and exosomes. Lipid nanoparticles have emerged as a promising non-immunogenic alternative to viral vectors, and are safe and effective (Zou et al.). One significant benefit to lipid nanoparticles is their 100nm size–compared to AAVs, which are 20-25 nm. Nanoparticles can also vary in size depending on the scientist's demands, while AAVs are unable to. This increased size allows lipid nanoparticles to carry a larger amount of CRISPR components to target cells (Kazemian et al.). However, additional research is necessary to engineer lipid nanoparticles to cross the BBB successfully (Han et al.). Lipid nanoparticles also readily degrade by the body after cargo release, which can reduce immunogenicity (Swingle et al.).

Additionally, non-viral approaches are arguably a safer procedure to use than viral approaches. While AAV has high risk to health effects (13 deaths have been reported from 1999 to 2023 due to high-dose AAV) (Salomonsson and Clelland), synthetic particles like lipid nanoparticles show a safer procedure due to readily degrading within cells. This further highlights the promise surrounding non-viral approaches, emphasizing their safety. Still, while lipid nanoparticles have a promising future, additional research is needed to overcome some of the limitations of this technology.

[2] Other approaches:

More promising alternatives for effective delivery of CRISPR to the CNS include small-molecule drugs, monoclonal antibodies, CAR T-cell therapy, and external means using ultrasound or light (Gao). Two key delivery systems are the intrathecal administration and the convection-enhanced delivery. Intrathecal administration (IT) is a process that involves directly delivering substances to the cerebrospinal fluid (CSF) within the spinal canal using a needle(NIH, *Definition of Intrathecal - NCI Dictionary of Cancer Terms - NCI*). Through this method, the BBB can be bypassed altogether. Convection-enhanced delivery (CED) is a method that delivers therapeutic agents to the CNS by directly infusing them into the brain or spinal cord under pressure (Mehta et al.). CED shows improved precision and efficacy in delivery to the CNS compared to viral vectors delivered systemically. The key differences between IT and CED are that IT targets the CSF and the surface of the brain, while CED targets the parenchymal tissue deep within the spinal cord. Moreover, external means can be used as well for CRISPR delivery. External means include ultrasound-mediated delivery, a remote-controlled technique that was successful in opening the BBB. This is positive for the future of gene therapies. Light-mediated delivery is also an area of research, using light-responsive materials to control genetic material release; these nanoparticles respond to wavelengths of light and can be very specific.

Table 2: Strategies to deliver CRISPR components into the CNS

CRISPR delivery strategies		**Expected outcome**	**Citations**
Viral vector approaches	Requirements for a successful viral vector	High transduction efficiency, safe, large capacity, small size	(Asmamaw Mengstie) (Salomonsson and Clelland)
	Delivery of CRISPR ribonucleoproteins	Regulates RNA processing and enzymatic activities	(Chen et al.)
Non-viral vector approaches	Lipid nano particles	Has large luggage size and is safe and effective	(Zou et al.) (Han et al.) (Swingle et al.)
	Intrathecal administration and the Convection-en	These methods can bypass the BBB	(Gao) (Mehta et al.)

	hanced delivery		

Immune cell-specific approaches for CRISPR treatment for MS

The pathogenesis of autoimmune diseases is commonly described as a loss of immune tolerance to self-proteins due to genetic and environmental factors. Since immune cells lose their ability to mount a suitable immune response, targeting immune cell-specific approaches (figure 3) for MS using CRISPR could also prove helpful for CNS diseases (Table 3). The main issue for autoimmune diseases is the autoreactivity by specific cells, which is when they stimulate a response against the body's own tissues. Specifically in MS, immune cells, which otherwise would have helped the immune system, are turned against it and cause inflammation in the brain.

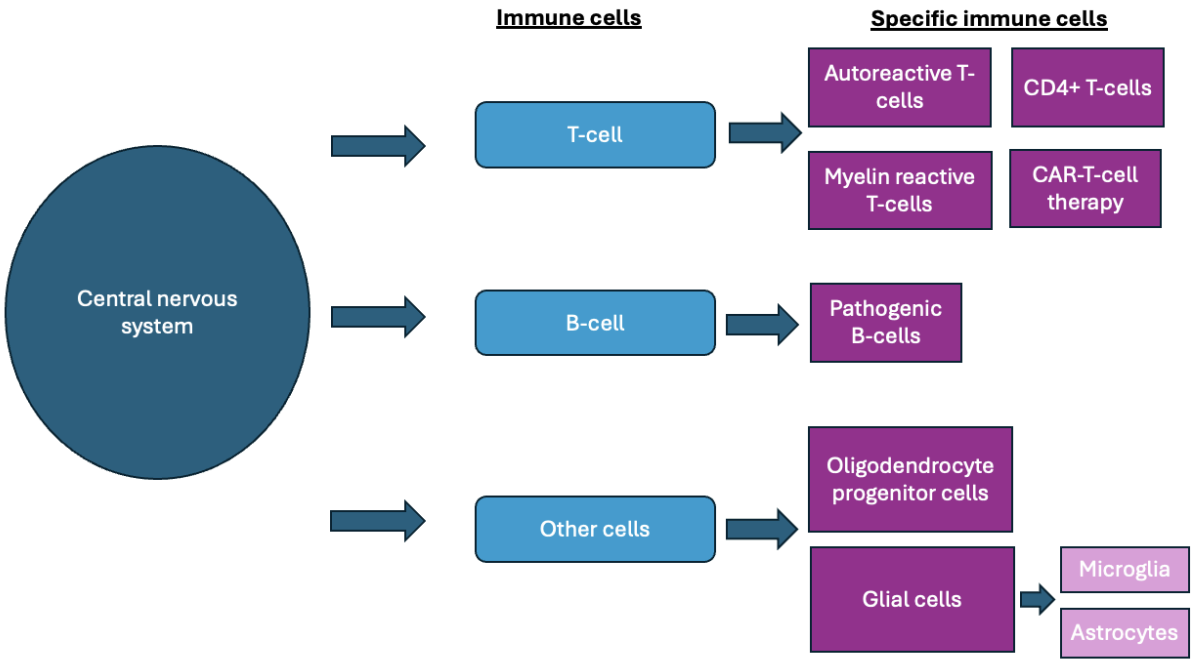

Figure 3: Specific immune cells in the CNS can be targeted to reduce demyelination and promote repair in nerve cells.

T-cell related approaches

A crucial immune cell example is the T-cell. T-cells in MS enter the CNS and recognize their target auto-antigen. They start by producing inflammatory cytokines which increase BBB permeability and then recruit other immune cells to inflammatory lesions (Charles A Janeway et

al.). The T-cells release chemicals that cause inflammation, damaging the myelin, nerve fibres, and myelin-making cells. In 1999, research by Madsen et al. demonstrated that adoptive transfers of CNS antigen-specific T-cells induce inflammation in the CNS (Madsen et al.). Additionally, the results of a later genome-wide study identified risk loci associated with immune cells for MS patients (Kawakami and Wekerle). This is important because autoreactivity forms the basis of autoimmune diseases. Editing autoreactive T-cells could be hugely beneficial, as it could reverse the harmful effects the T-cells have on the CNS. Instead of causing inflammation, T-cells could be edited to enable them to promote myelin repair and reduce inflammation. There's another type of T-cell at play in MS, CD4+ T-cells (Ma et al.). CD4+ T-cells have been found to play an essential role in autoimmune responses. In 1986, Mosmann *et al.,* proposed that CD4+ T-cells could differentiate into two functionally opposing subsets, Th1 and Th2 cells (Mosmann et al.).

Within the context of MS, Th1 cells activate macrophages (a type of white blood cell that surrounds and kills microorganisms), leading to CNS migration and, consequently, a pro-inflammatory effect. This is crucial as it shows the direct immune cell used will cause brain inflammation, which can worsen MS because autoreactive T-cells will prey on the patient's own immune system. CD4+ T-cells create a good target to edit. On the other hand, Th2 cells work in opposition to Th1 cells; they have negative feedback on the differentiation of Th1 cells and therefore have an anti-inflammatory action (Oreja-Guevara et al.). Since Th1 and Th2 cells work oppositely, the autoimmune inflammatory response in MS is directly related to the imbalance between Th1/Th2. This echoes the potential that T-cells can be a target for MS therapy. Since T-cells play such a significant role in the development of MS, reversing the harmful effects of autoreactivity in T-cells can be a game-changer in MS treatment. Given T-cells' impact on MS, research to reprogram CD4+ T-cells has aimed to decrease their autoreactivity. In a paper by Colson *et al.,* genetically edited CD4+ effector T-cells in mice were shown to not only prevent autoimmune neuroinflammation before onset, but also stop the MS progression (Colson et al.)

Another approach targets autologous myelin-reactive T-cells, utilizing the patient's own immune cells. Early trials *in vivo* reported a decrease in pathogenic T-cells and lower toxicity. There was also a reduction of lesion count (a biological marker of MS), relapse rate, and neurological disability (Colson et al.).

CAR T-cell therapy is also a new and successful method for genetically modifying a patient's own immune cells (T-cells) to target and eliminate harmful B-cells contributing to the disease. This method is beneficial because it can reduce the rate of host-versus-graft disease, a context in which the cells delivered to a patient are rejected by their own immune system (Sanber et al.). This is a relatively new technology, but it shows promise. CAR T-cells can enter the CNS and attack immune cells, particularly to deplete B-cells. In a study by Fischbach *et al.*, CD19 CAR-T cell therapy demonstrated tolerable short-term safety in two patients with MS. Additionally, CAR

T-cells showed a safe entry and expansion in the CSF without neurotoxicity, supporting the previously tested claim that T-cells can be edited for therapeutic benefit (Fischbach et al.). Overall, the various approaches that target T-cells are crucial in the context of MS, given the pivotal role T-cells play in the development of MS within the CNS.

B-cell related approaches

Although T-cells are typically considered a causative agent of MS, new research studies have shown evidence that B-cells also contribute to MS (Comi et al.). During MS, T-cells in MS activate B-cells, this process results in the recruitment of other immune system cells to also attack the myelin sheath (McLaughlin and Wucherpfennig). While B-cells are not normally found inside the CNS or spinal cord, in MS, B-cells mistakenly enter the CNS and cause damage by generating autoreactive antibodies that target myelin, resulting in MS (McLaughlin and Wucherpfennig). Additionally, B-cells in MS also secrete proinflammatory cytokines, which further contributes to CNS autoimmunity (Ma et al.). Overall, this research suggests that B-cells could also contribute to not only MS relapses, but also the progression of the disease.

Recently, there has been a push within the scientific community to develop treatment methods to modify the function of autoreactive B-cells, given that the focus was previously concentrated on T-cells for their involvement in the progression of MS. Developed in 2008, a drug named rituximad has shown efficacy in the control of MS by targeting B-cells specifically. The mechanism of action of rituximab involves targeting the CD20+ molecule (a protein found on the surface of B cells crucial for B cell activation and differentiation), leading to direct killing of B-cells (Chisari et al.). There are other drugs which perform similarly, including: ocrelizumab, ofatumumab and ublituximab which target pathogenic B-cells (Milo). However, rituximab has shown incredibly promising results. In a study by Hauser et al., the use of rituximab in treating MS patients showed markedly reduced MRI evidence of MS disease activity, and steeply reduced the clinical relapse rate (Hauser et al.) There are also novel approaches being developed also directed at B-cells. Using different genetic methods, there is research focused on modifying B-cells that can differentiate into memory B-cells (Wang et al.). This is an exciting approach, as it could prove helpful for providing durable immunity against mutating antigens involved in MS. Modifying genetics of B cells could be an area used for further research due to its already shown success in disease slowing drugs now.

Cells that suppress neuroinflammation and promote repair

Another approach to treat MS has been focused on targeting cells that suppress neuroinflammation and promote repair. One example is the GAT107 drug, a strong positive allosteric modulator (a substance that enhances the activity of a receptor by binding to a site different from the main binding site) (Mizrachi et al.). Research on GAT107 by Mizrachi et al.,

described that immune cells altered by GAT107 treatment resulted in a significant reduction in macrophages, dendritic cells and B cells. The disease severity was significantly reduced by 70% and was linked to the reduction of neuroinflammation in the CNS. Specifically, there was a 37% decrease in macrophages, 31% decrease of dendritic cells. There was also a 2.8-fold increase in the secretion of anti-inflammatory cytokines IL-10 (Mizrachi et al.). This is important because the reduction of these cells can reduce neuroinflammation, which significantly contributes to the progression of MS.

Another cell that promotes repair within the CNS are Oligodendrocyte progenitor cells (OPCs) which have been recognized for their ability for pro-regenerative strategies (Zveik et al.). Data shows that OPCs can produce myelin and respond to inflammation (Zveik et al.). This is important because (explain why this matters clearly in relation to MS). OPCs are activated during demyelination, which can occur as a result of autoimmunity. Next, the OPCs differentiate into myelinating oligodendrocytes (McCurry). In this form, the cells have a hostile lesion environment, when there is much myelin debris. Hence, this is not sufficient for repair needs in MS, so further research must still be made.

Lastly, glial cells, particularly microglia and astrocytes, play crucial roles in suppressing neuroinflammation and promoting repair (Han et al.). Microglia and astrocytes can release anti-inflammatory factors like IL-10 and BDNF that reduce the inflammatory response leading to MS. Likewise, microglia can transition from a pro-inflammatory state to an anti-inflammatory state (Gao et al.). They do this by communicating with surrounding cells and releasing anti-inflammatory cytokines to cause a phenotypic shift. This is optimal for MS treatment because inflammation is a key obstacle to fight against and microglia can beneficially transition. Currently, more than 16 treatments for MS have been approved by the US Food and Drug Administration, including Disease Modifying Therapies (DMTs) which work by targeting the immune system's inflammatory response, therefore decreasing the number and severity of relapses and slow MS progression. DMTs include interferons and Glatiramer acetate (Zveik et al.). Also, oligodendrocytes are glial cells that play a vital role in maintaining the myelin sheath (Chamberlain et al.). However, in MS, oligodendrocytes become dysfunctional because the immune system mistakenly attacks them and their myelin sheaths. This leads to myelin damage, and the subsequent formation of lesions in the CNS associated with MS (López-Muguruza and Matute). Focus on repairing the dysfunction of oligodendrocytes using CRISPR has been an active area of research, particularly focusing on the use of genetic editing to restore the function of oligodendrocytes, thereby restoring repair of the myelin sheath (Azeez et al.). The overall goal of these approaches is to reverse the autoimmunity implicated in MS, and ultimately, treat the disease.

Table 3: Immune cell-specific approaches for CRISPR treatment for MS

Immune cell-specific approaches		Expected outcome	Reference
T-cell-related approaches	Editing autoreactive T-cells	Instead of demyelination, T-cells can be edited to promote repair	(Madsen et al.) (Mosmann et al.)
	Editing CD4+ T-cells to 2 opposing subsets: Th1 and Th2	They have anti-inflammatory action so can reverse harm from autoreactive T-cell	(Oreja-Guevara et al.) (Colson et al.)
	Myelin reactive T-cells	Using a patient's own immune cells can treat MS	(Colson et al.)
	CAR- T-cell therapy	Genetically modifying a patient's T-cells to target harmful B-cells	(Sanber et al.) (Fischbach et al.)
B-cell related approaches	Edit B-cells so they don't attack CNS	B-cells in MS generate autoreactive antibodies that target myelin	(Comi et al.) (McLaughlin and Wucherpfennig).
	Examples of B-cell targeted drugs	Rituximad has shown efficacy in the control of MS by targeting B-cells	(Chisari et al.) (Hauser et al.)
Cells that suppress neuroinflammation and promote repair	GAT-107 drug that targets immune cells	Showed a significant reduction in macrophages, dendritic cells and B cells, which reduce neuroinflammation	(Mizrachi et al.)
	Oligodendrocyte progenitor cells	They can promote remyelination	(Zveik et al.)
	Glial cells	Communicating with surrounding cells and releasing anti-inflammatory cytokines for repair	(Gao et al.) (Chamberlain et al.) (López-Muguruza and Matute)

Conclusion & Future Directions

In summary, challenges and strategies vary widely for delivering CRISPR tools to immune cells in the CNS involved in MS. Biological barriers include the blood-brain barrier, immune cell heterogeneity and off-target effects. It is important for these barriers to be overcome because otherwise the harmful effects to our body could be long term and detrimental. Our body has its own natural immune system designed for its specific purpose, therefore trying to break through these biological barriers for CRISPR treatment is unwelcome, so the overcoming of these biological barriers is crucial. Technical barriers include the limitations to viral vectors, immunogenicity, and the efficiency of gene editing. This is important because inefficient delivery of CRISPR could lead to a redundant treatment. We need to secure an optimal carriage for this treatment first, before its treatment effects are discussed. These barriers are in crucial need to be overcome because this could be a cure for many autoimmune diseases like MS. Some strategies are still being researched, like viral and non-viral approaches and immune cell specific approaches. But without the overcoming of these barriers, they will not be effective.

The future of gene editing medical therapies for MS is in reach. Specifically, an increased push for personalized medicine focused on treating the particular facets of an individual's MS phenotype. This is important because immune cell heterogeneity will prevent similar MS patients to successfully get treated by the same CRISPR components. This is a noticeable obstacle as it shows how even if CRISPR is delivered successfully into the CNS, CRISPR may still not be effective in its treatment. Solutions to implement conditions for safety, effectiveness, and accuracy will need to be thoroughly designed and rigorously tested to even ensure proper delivery of CRISPR components to the CNS. The benefit of this type of research and development is that it extends beyond MS. There are several other autoimmune diseases that require similar technology to be designed for similar therapeutic processes. Therefore, this research has the opportunity to open the door for the treatment of other autoimmune diseases. And, perhaps, infectious disease, regenerative medicine, and tissue engineering more broadly (Azeez et al.)

Overall, MS is a disease that is in crucial need for treatment options. Statistically, around the world, a diagnosis of MS is made every 5 minutes (Modglin). This alarming statistic pushes an urgency for treatment. Current strategies are not enough to reduce the harming consequences of MS to the population. While MS can be managed partially by therapeutics, this is not a full treatment for the disease. Treatments like DMTs only slow down the disease progression, not stop it completely, relapses are also common (Tilling et al.). Additionally, many patients still succumb to the disease in time. The potential for CRISPR therapies is compelling as it transitions MS from a disease that can only be managed and slowed, to one that could be treated outright. Thereby changing the lives of many individuals worldwide.

By emphasizing the biological and technological advancements necessary for delivery of CRISPR components to the CNS, this paper highlights strategies to overcome these challenges. These advancements will likely require collaboration between a multitude of professions, ranging from biologists and immunologists to bioengineers. However, through successful collaboration and technological advancements, it is possible that, with the prioritization of safety and ethics, the lives of 2.3 million people worldwide affected by MS could be improved.

Acknowledgement
I am thankful to Dr. Lalita Limaye for mentoring support.

References

1. Alsaiari, Shahad K., et al. 'CRISPR–Cas9 Delivery Strategies for the Modulation of Immune and Non-Immune Cells'. *Nature Reviews Materials*, vol. 10, no. 1, Jan. 2025, pp. 44–61. *www.nature.com*, https://doi.org/10.1038/s41578-024-00725-7.
2. Arneth, Borros M. 'Impact of B Cells to the Pathophysiology of Multiple Sclerosis'. *Journal of Neuroinflammation*, vol. 16, no. 1, Jun. 2019, p. 128. *BioMed Central*, https://doi.org/10.1186/s12974-019-1517-1.
3. Asmamaw Mengstie, Misganaw. 'Viral Vectors for the in Vivo Delivery of CRISPR Components: Advances and Challenges'. *Frontiers in Bioengineering and Biotechnology*, vol. 10, May 2022, p. 895713. *PubMed Central*, https://doi.org/10.3389/fbioe.2022.895713.
4. Azeez, Sarkar Sardar, et al. 'Advances in CRISPR-Cas Technology and Its Applications: Revolutionising Precision Medicine'. *Frontiers in Genome Editing*, vol. 6, Dec. 2024, p. 1509924. *PubMed Central*, https://doi.org/10.3389/fgeed.2024.1509924.

5. Barua, Neil U., et al. ‘Convection‐Enhanced Drug Delivery to the Brain: Therapeutic Potential and Neuropathological Considerations’. *Brain Pathology*, vol. 24, no. 2, Sep. 2013, pp. 117–27. *PubMed Central*, https://doi.org/10.1111/bpa.12082.
6. Blauth, Kevin, et al. ‘Antibodies Produced by Clonally Expanded Plasma Cells in Multiple Sclerosis Cerebrospinal Fluid Cause Demyelination of Spinal Cord Explants’. *Acta Neuropathologica*, vol. 130, no. 6, Dec. 2015, pp. 765–81. *Springer Link*, https://doi.org/10.1007/s00401-015-1500-6.
7. Chamberlain, Kelly A., et al. ‘Oligodendrocyte Regeneration: Its Significance in Myelin Replacement and Neuroprotection in Multiple Sclerosis’. *Neuropharmacology*, vol. 110, no. Pt B, Nov. 2016, pp. 633–43. *PubMed Central*, https://doi.org/10.1016/j.neuropharm.2015.10.010.
8. Charles A Janeway, Jr, et al. ‘The Production of Armed Effector T Cells’. *Immunobiology: The Immune System in Health and Disease. 5th Edition*, Garland Science, 2001. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK27118/.
9. Chen, Kai, et al. ‘Engineering Self-Deliverable Ribonucleoproteins for Genome Editing in the Brain’. *Nature Communications*, vol. 15, no. 1, Feb. 2024, p. 1727. *www.nature.com*, https://doi.org/10.1038/s41467-024-45998-2.
10. Chisari, Clara Grazia, et al. ‘Rituximab for the Treatment of Multiple Sclerosis: A Review’. *Journal of Neurology*, vol. 269, no. 1, Jan. 2022, pp. 159–83. *PubMed*, https://doi.org/10.1007/s00415-020-10362-z.
11. Colson, Tyler R., et al. ‘Regulatory T Cells Epigenetically Reprogrammed from Autoreactive Effector T Cells Mitigate Established Autoimmunity’. *bioRxiv*, Jul. 2025, p. 2025.07.24.665398. *PubMed Central*, https://doi.org/10.1101/2025.07.24.665398.

12. Comi, Giancarlo, et al. ‘The Role of B Cells in Multiple Sclerosis and Related Disorders’. *Annals of Neurology*, vol. 89, no. 1, Jan. 2021, pp. 13–23. *PubMed Central*, https://doi.org/10.1002/ana.25927.
13. Doshi, Anisha, and Jeremy Chataway. ‘Multiple Sclerosis, a Treatable Disease’. *Clinical Medicine*, vol. 16, no. Suppl 6, Dec. 2016, pp. s53–59. *PubMed Central*, https://doi.org/10.7861/clinmedicine.16-6s-s53.
14. Engelhardt, Britta, et al. ‘Multiple Sclerosis: Immunopathological Heterogeneity and Its Implications’. *European Journal of Immunology*, vol. 52, no. 6, Jun. 2022, pp. 869–81. *DOI.org (Crossref)*, https://doi.org/10.1002/eji.202149757.
15. Fassi, Daniel El, et al. ‘Ulcerative Colitis Following B Lymphocyte Depletion with Rituximab in a Patient with Graves’ Disease’. *Gut*, vol. 57, no. 5, May 2008, p. 714, https://doi.org/10.1136/gut.2007.138305.
16. Fischbach, Felix, et al. ‘CD19-Targeted Chimeric Antigen Receptor T Cell Therapy in Two Patients with Multiple Sclerosis’. *Med*, vol. 5, no. 6, Jun. 2024, pp. 550-558.e2. *ScienceDirect*, https://doi.org/10.1016/j.medj.2024.03.002.
17. Gao, Chao, et al. ‘Microglia in Neurodegenerative Diseases: Mechanism and Potential Therapeutic Targets’. *Signal Transduction and Targeted Therapy*, vol. 8, no. 1, Sep. 2023, p. 359. *www.nature.com*, https://doi.org/10.1038/s41392-023-01588-0.
18. Gao, Zhenghong. ‘Strategies for Enhanced Gene Delivery to the Central Nervous System’. *Nanoscale Advances*, vol. 6, no. 12, pp. 3009–28. *PubMed Central*, https://doi.org/10.1039/d3na01125a.

19. Gonçalves, Manuel AFV. 'Adeno-Associated Virus: From Defective Virus to Effective Vector'. *Virology Journal*, vol. 2, May 2005, p. 43. *PubMed Central*, https://doi.org/10.1186/1743-422X-2-43.
20. Gray, Steven J., et al. 'Viral Vectors and Delivery Strategies for CNS Gene Therapy'. *Therapeutic Delivery*, vol. 1, no. 4, Oct. 2010, p. 517. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.4155/tde.10.50.
21. Guo, Congting, et al. 'Off-Target Effects in CRISPR/Cas9 Gene Editing'. *Frontiers in Bioengineering and Biotechnology*, vol. 11, Mar. 2023, p. 1143157. *PubMed Central*, https://doi.org/10.3389/fbioe.2023.1143157.
22. Han, Jialin, et al. 'The Roles of Microglia and Astrocytes in Neuroinflammation of Alzheimer's Disease'. *Frontiers in Neuroscience*, vol. 19, May 2025, p. 1575453. *PubMed Central*, https://doi.org/10.3389/fnins.2025.1575453.
23. Hunt, John Murray Topp, et al. 'Unintended CRISPR-Cas9 Editing Outcomes: A Review of the Detection and Prevalence of Structural Variants Generated by Gene-Editing in Human Cells'. *Human Genetics*, vol. 142, no. 6, 2023, pp. 705–20. *PubMed Central*, https://doi.org/10.1007/s00439-023-02561-1.
24. Ishino, Y, et al. 'Nucleotide Sequence of the Iap Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in Escherichia Coli, and Identification of the Gene Product.' *Journal of Bacteriology*, vol. 169, no. 12, Dec. 1987, pp. 5429–33. *PubMed Central*, https://doi.org/10.1128/jb.169.12.5429-5433.1987.
25. Kallimasioti-Pazi, Eirini M., et al. 'Heterochromatin Delays CRISPR-Cas9 Mutagenesis but Does Not Influence the Outcome of Mutagenic DNA Repair'. *PLoS Biology*, vol. 16,

no. 12, Dec. 2018, p. e2005595. *PubMed Central*, https://doi.org/10.1371/journal.pbio.2005595.

26. Kang, Lin, et al. 'AAV Vectors Applied to the Treatment of CNS Disorders: Clinical Status and Challenges'. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, vol. 355, Mar. 2023, pp. 458–73. *PubMed*, https://doi.org/10.1016/j.jconrel.2023.01.067.
27. Kang, Xianhui, et al. 'Progresses, Challenges, and Prospects of CRISPR/Cas9 Gene-Editing in Glioma Studies'. *Cancers*, vol. 15, no. 2, Jan. 2023, p. 396. *PubMed Central*, https://doi.org/10.3390/cancers15020396.
28. Kawakami, Naoto, and Hartmut Wekerle. 'Life History of a Brain Autoreactive T Cell: From Thymus through Intestine to Blood-Brain Barrier and Brain Lesion'. *Neurotherapeutics*, vol. 21, no. 6, Sep. 2024, p. e00442. *PubMed Central*, https://doi.org/10.1016/j.neurot.2024.e00442.
29. Kazemian, Pardis, et al. 'Lipid-Nanoparticle-Based Delivery of CRISPR/Cas9 Genome-Editing Components'. *Molecular Pharmaceutics*, vol. 19, no. 6, Jun. 2022, pp. 1669–86. *PubMed Central*, https://doi.org/10.1021/acs.molpharmaceut.1c00916.
30. Kendirli, Arek, et al. 'A Genome-Wide in Vivo CRISPR Screen Identifies Essential Regulators of T Cell Migration to the CNS in a Multiple Sclerosis Model'. *Nature Neuroscience*, vol. 26, no. 10, 2023, pp. 1713–25. *PubMed Central*, https://doi.org/10.1038/s41593-023-01432-2.
31. Kim, Segi, et al. 'Genome Editing of Immune Cells Using CRISPR/Cas9'. *BMB Reports*, vol. 54, no. 1, Jan. 2021, pp. 59–69. *PubMed Central*, https://doi.org/10.5483/BMBRep.2021.54.1.245.

32. Kumar, Rajnish, et al. ‘DeePred-BBB: A Blood Brain Barrier Permeability Prediction Model With Improved Accuracy’. *Frontiers in Neuroscience*, vol. 16, 2022, p. 858126. *PubMed*, https://doi.org/10.3389/fnins.2022.858126.
33. López-Muguruza, Eneritz, and Carlos Matute. ‘Alterations of Oligodendrocyte and Myelin Energy Metabolism in Multiple Sclerosis’. *International Journal of Molecular Sciences*, vol. 24, no. 16, Aug. 2023, p. 12912. *pmc.ncbi.nlm.nih.gov*, https://doi.org/10.3390/ijms241612912.
34. Ma, Hong, et al. ‘Correction of a Pathogenic Gene Mutation in Human Embryos’. *Nature*, vol. 548, no. 7668, 7668, Aug. 2017, pp. 413–19. *www.nature.com*, https://doi.org/10.1038/nature23305.
35. Madsen, L. S., et al. ‘A Humanized Model for Multiple Sclerosis Using HLA-DR2 and a Human T-Cell Receptor’. *Nature Genetics*, vol. 23, no. 3, Nov. 1999, pp. 343–47. *PubMed*, https://doi.org/10.1038/15525.
36. Malkani, Shaila. *The Forefront of a Revolution: The Applications of CRISPR-Cas9 Technology to Treat Multiple Sclerosis*. 2022.
37. McCurry, Justin. ‘Scientist Who Gene-Edited Babies Is Back in Lab and “Proud” of Past Work despite Jailing’. *The Guardian*, 1 Apr. 2024. Science. *The Guardian*, https://www.theguardian.com/science/2024/apr/01/crispr-cas9-he-jiankui-genome-gene-editing-babies-scientist-back-in-lab.
38. McLaughlin, Katherine A., and Kai W. Wucherpfennig. ‘B Cells and Autoantibodies in the Pathogenesis of Multiple Sclerosis and Related Inflammatory Demyelinating Diseases’. *Advances in Immunology*, vol. 98, 2008, pp. 121–49. *PubMed Central*, https://doi.org/10.1016/S0065-2776(08)00404-5.

39. Mehta, A. M., et al. ‘Convection-Enhanced Delivery’. *Neurotherapeutics*, vol. 14, no. 2, Apr. 2017, pp. 358–71. *PubMed Central*, https://doi.org/10.1007/s13311-017-0520-4.
40. Meneghini, Vasco, et al. ‘Delivery Platforms for CRISPR/Cas9 Genome Editing of Glial Cells in the Central Nervous System’. *Frontiers in Genome Editing*, vol. 3, Mar. 2021. *Frontiers*, https://doi.org/10.3389/fgeed.2021.644319.
41. Milo, Ron. ‘Therapies for Multiple Sclerosis Targeting B Cells’. *Croatian Medical Journal*, vol. 60, no. 2, Apr. 2019, pp. 87–98. *PubMed Central*, https://doi.org/10.3325/cmj.2019.60.87.
42. Mizrachi, Tehila, et al. ‘Suppression of Neuroinflammation by an Allosteric Agonist and Positive Allosteric Modulator of the A7 Nicotinic Acetylcholine Receptor GAT107’. *Journal of Neuroinflammation*, vol. 18, no. 1, Apr. 2021, p. 99. *PubMed*, https://doi.org/10.1186/s12974-021-02149-4.
43. Modglin, Cathy Habas, Lindsay. ‘Multiple Sclerosis Statistics 2025’. *The Checkup*, 2 Mar. 2022, https://www.singlecare.com/blog/news/multiple-sclerosis-statistics/.
44. Mosmann, T. R., et al. ‘Two Types of Murine Helper T Cell Clone. I. Definition According to Profiles of Lymphokine Activities and Secreted Proteins’. *Journal of Immunology (Baltimore, Md.: 1950)*, vol. 136, no. 7, Apr. 1986, pp. 2348–57.
45. Murray, Christopher J. L. ‘The Global Burden of Disease Study at 30 Years’. *Nature Medicine*, vol. 28, no. 10, Oct. 2022, pp. 2019–26. *DOI.org (Crossref)*, https://doi.org/10.1038/s41591-022-01990-1.
46. NIH. ‘Definition of Chromatin - NCI Dictionary of Cancer Terms - NCI’. nciAppModulePage. *NIH- National Cancer Institute*, 2 Feb. 2011,

https://www.cancer.gov/publications/dictionaries/cancer-terms/def/chromatin. nciglobal,ncienterprise.

———. *Definition of Intrathecal - NCI Dictionary of Cancer Terms - NCI*. nciAppModulePage. 2 Feb. 2011, https://www.cancer.gov/publications/dictionaries/cancer-terms/def/intrathecal. nciglobal,ncienterprise.

47. Oreja-Guevara, Celia, et al. 'TH1/TH2 Cytokine Profile in Relapsing-Remitting Multiple Sclerosis Patients Treated with Glatiramer Acetate or Natalizumab'. *BMC Neurology*, vol. 12, Sep. 2012, p. 95. *PubMed Central*, https://doi.org/10.1186/1471-2377-12-95.
48. Pandit, Rucha, et al. 'The Blood-Brain Barrier: Physiology and Strategies for Drug Delivery'. *Advanced Drug Delivery Reviews*, vols 165–166, 2020, pp. 1–14. *PubMed*, https://doi.org/10.1016/j.addr.2019.11.009.
49. Peterson, Craig L. 'Heterochromatin: Hiding from the Remodeling Machines'. *Molecular Cell*, vol. 84, no. 17, Sep. 2024, pp. 3167–69. *ScienceDirect*, https://doi.org/10.1016/j.molcel.2024.08.012.
50. Proulx, Steven T., and Britta Engelhardt. 'Central Nervous System Zoning: How Brain Barriers Establish Subdivisions for CNS Immune Privilege and Immune Surveillance'. *Journal of Internal Medicine*, vol. 292, no. 1, Jul. 2022, pp. 47–67. *PubMed*, https://doi.org/10.1111/joim.13469.
51. Ransohoff, Richard M., and Melissa A. Brown. 'Innate Immunity in the Central Nervous System'. *The Journal of Clinical Investigation*, vol. 122, no. 4, Apr. 2012, pp. 1164–71. *PubMed Central*, https://doi.org/10.1172/JCI58644.

52. Salomonsson, Sally E., and Claire D. Clelland. ‘Building CRISPR Gene Therapies for the Central Nervous System A Review’. *JAMA Neurology*, vol. 81, no. 3, Mar. 2024, pp. 283–90. *PubMed Central*, https://doi.org/10.1001/jamaneurol.2023.4983.
53. Sanber, Khaled, et al. ‘Graft-versus-Host Disease Risk after Chimeric Antigen Receptor T-Cell Therapy: The Diametric Opposition of T Cells’. *British Journal of Haematology*, vol. 195, no. 5, Dec. 2021, pp. 660–68. *PubMed*, https://doi.org/10.1111/bjh.17544.
54. Shah, Neal. *Implantable Intrathecal Drug Delivery System - StatPearls - NCBI Bookshelf*. https://www.ncbi.nlm.nih.gov/books/NBK538237/. Accessed 17 Sep. 2025.
55. Swingle, Kelsey L., et al. ‘Lipid Nanoparticle-Mediated Delivery of mRNA Therapeutics and Vaccines’. *Trends in Molecular Medicine*, vol. 27, no. 6, Jun. 2021, pp. 616–17. *PubMed*, https://doi.org/10.1016/j.molmed.2021.03.003.
56. Tilling, Kate, et al. ‘Background’. *Modelling Disease Progression in Relapsing–Remitting Onset Multiple Sclerosis Using Multilevel Models Applied to Longitudinal Data from Two Natural History Cohorts and One Treated Cohort*, NIHR Journals Library, 2016. *www.ncbi.nlm.nih.gov*, https://www.ncbi.nlm.nih.gov/books/NBK395613/.
57. Ule, Jernej. *Ribonucleoprotein - an Overview | ScienceDirect Topics*. https://www.sciencedirect.com/topics/neuroscience/ribonucleoprotein. Accessed 29 Aug. 2025.
58. Wang, Jiang-Hui, et al. ‘Adeno-Associated Virus as a Delivery Vector for Gene Therapy of Human Diseases’. *Signal Transduction and Targeted Therapy*, vol. 9, no. 1, Apr. 2024, p. 78. *www.nature.com*, https://doi.org/10.1038/s41392-024-01780-w.

59. Wu, Di. *The Blood–Brain Barrier: Structure, Regulation and Drug Delivery | Signal Transduction and Targeted Therapy*. 25 May 2023, https://www.nature.com/articles/s41392-023-01481-w.

60. Yong, V. Wee. 'Microglia in Multiple Sclerosis: Protectors Turn Destroyers'. *Neuron*, vol. 110, no. 21, Nov. 2022, pp. 3534–48. *www.cell.com*, https://doi.org/10.1016/j.neuron.2022.06.023.

61. Zou, Yan, et al. 'Blood-Brain Barrier–Penetrating Single CRISPR-Cas9 Nanocapsules for Effective and Safe Glioblastoma Gene Therapy'. *Science Advances*, vol. 8, no. 16, Apr. 2022, p. eabm8011. *DOI.org (Crossref)*, https://doi.org/10.1126/sciadv.abm8011.

62. Zveik, Omri, et al. 'The Interplay of Inflammation and Remyelination: Rethinking MS Treatment with a Focus on Oligodendrocyte Progenitor Cells'. *Molecular Neurodegeneration*, vol. 19, no. 1, Jul. 2024, p. 53. *BioMed Central*, https://doi.org/10.1186/s13024-024-00742-8.