Recover, repair or restrict?

New therapeutic perspectives for MS patients

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Samenvatting in gewone taal

Multiple sclerose (MS) is een chronische ziekte waarbij het afweersysteem het centrale zenuwstelsel (CZS) aanvalt. Hierdoor wordt de beschermende laag rondom de zenuwen beschadigd, waardoor de zenuwen zelf ook beschadigd kunnen raken. Patiënten krijgen hierdoor klachten, bijvoorbeeld uitval van lichaamsdelen. De medicijnen die nu beschikbaar zijn voor MS-patiënten, remmen het afweersysteem af. Maar deze zijn niet voor iedereen werkzaam. Daarom wordt er veel onderzoek gedaan naar nieuwe behandelingen. Deze kunnen op basis van hun doel grofweg verdeeld worden in drie categorieën: herstel, reparatie en het beperken van de schade aan het CZS.

Herstel

Stamceltherapie (aHSCT) is één van de herstellende behandelingen die onderzocht worden. Het doel van aHSCT is om het hele afweersysteem te verwijderen en weer op te bouwen. Vanuit de stamcellen ontstaan nieuwe afweercellen, waardoor het afweersysteem hersteld wordt. Door deze nieuwe variant wordt het CZS niet meer aangevallen, waardoor patiënten geen nieuwe klachten ontwikkelen.

Poeptransplantaties zorgen voor herstel van de darmflora: de bacteriën in de dikke darm die grote invloed hebben op onze gezondheid. Bij patiënten met MS is de samenstelling van deze bacteriën niet meer in balans, wat mogelijk bijdraagt aan het ontwikkelen van MS-klachten. Tijdens de behandeling wordt de poep van een gezonde donor getransplanteerd naar een patiënt. Omdat in de poep een afdruk zit van de gezonde darmflora van de donor, worden gezonde bacteriën getransplanteerd. Onderzoekers hebben aangetoond dat de samenstelling van de darmflora van een patiënt hierdoor verbeterd wordt. Of dit bij MS-patiënten ook voor minder klachten zorgt, is nog niet duidelijk.

Reparatie

Omdat het CZS de schade aan de zenuwen niet goed kan herstellen, krijgen MS-patiënten steeds meer klachten. De beschermende laag om de zenuwen kan wél gerepareerd worden, wat de schade aan de zenuwen zelf voorkomt. Onderzoekers hebben ontdekt dat specifieke medicijnen hier een handje bij kunnen helpen door de cellen te activeren die de beschermende laag maken. Er wordt nog onderzoek gedaan om te bepalen of dit daadwerkelijk als behandeling gebruikt kan worden.

Beperken van de schade

Een andere strategie is het verkleinen van de kans op beschadiging. Dit kan door de productie van schadelijke stoffen te verminderen. Onderzoekers hebben aangetoond dat sommige medicijnen ervoor zorgen dat er bij patiënten minder schade in de hersenen werd aangericht. Patiënten bleken na de behandeling ook minder last hebben van MS-klachten. Hoe dit precies werkt? Dat is nog niet helemaal duidelijk en moet blijken uit toekomstige onderzoeken.

Andere mogelijkheden

Ook leefstijl heeft een grote invloed op het verloop van MS. Zo hebben onderzoekers aangetoond dat gezonde voeding en beweging belangrijk is voor MS-patiënten. Dit kan onder andere het afweersysteem afremmen, zenuwen beschermen en beschadigde verbindingen in het CZS herstellen. Uit de resultaten van een Nederlands leefstijlonderzoek moet blijken bij wie en in welke mate leefstijlverandering van belang is om MS af te remmen.

In het kort wordt er op dit moment veel onderzoek gedaan naar nieuwe manieren om MS-patiënten te behandelen. Vervolgonderzoek moet uitwijzen of deze therapieën ingezet kunnen worden om de ziekte nog verder af te remmen of misschien wel te stoppen.

Abstract

Up until today, the treatment of multiple sclerosis (MS) patients relies on immune modulation. A deeper understanding of MS pathogenesis has generated new perspectives on targeting the cause and consequences in the clinic. Therapies in this new generation can be roughly divided into three categories based on their mechanism of action: recovery, repair, and restriction. Recovering therapies focus on restoring balance in imbalanced systems, such as the immune system itself or the intestinal microbiota. Re-introducing balance leads to indirect amelioration of the autoreactive processes. Therapies inducing repair address the consequence of the intolerant immune response: damage to the central nervous system. Since this is associated with the development of symptoms, repair reduces MS-related impairments. Restricting therapies aim to prevent damage to neuronal fibers, thus preventing symptoms.

In addition to the therapies focusing on a specific aspect of pathogenesis, the holistic approach to MS management has gained attention over the years. Specifically, lifestyle adaptations have been shown to be effective in multiple ways, including downregulation of the autoreactive response and neuronal protection.

The new generation of MS therapies emphasizes the importance of a look beyond immune modulation. Counteracting the autoreactive response in a multifaceted way will more effectively inhibit disease progression.

Abbreviations

- BBB: blood-brain barrier
- BDNF: brain-derived neurotrophic factor
- CNS: central nervous system
- DMT: disease-modifying therapy
- FMT: fecal microbiota transplantation
- HSC: hematopoietic stem cell
- HSCT: hematopoietic stem cell transplantation
- IL: interleukin
- MS: multiple sclerosis
- MSC: mesenchymal stem cell
- NK: natural killer
- PA: physical activity
- PD-1: programmed cell death-1 protein
- PMS: progressive MS
- PPMS: primary progressive MS
- RRMS: relapsing remitting MS
- SPMS: secondary progressive MS
- TCR: T cell receptor

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1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease, characterized by an immune response against the myelin sheath surrounding neurons in the central nervous system (CNS). Although the exact cause of disease onset remains unknown, a variety of genetic, environmental, and infectious factors are involved in MS development.

Following damage to neuronal fibers underlying the affected myelin sheath, patients can experience a wide variety of symptoms, depending on the location of the damage [1]. Common exacerbations of the disease include loss of vision, muscle weakness, and fatigue. In most patients, these relapses are followed by periods of recovery. This manifestation of the disease is known as *relapsing remitting* MS (RRMS). With time, a substantial part of RRMS patients experiences a reduced recovery potential. This leads to a buildup of residual damage and worsening of symptoms, also known as *secondary progressive* MS (SPMS). In some, the progression of the disease is apparent directly from the onset, identified as *primary progressive* MS (PPMS) [2].

1.1. The autoimmune response

The autoimmune response in MS patients is characterized by the migration of pro-inflammatory $CD4^+$ T cells crossing the blood-brain barrier (BBB). When describing the subsequent immune response in the CNS, a distinction should be made between RRMS and progressive MS (PMS).

1.1.1. RRMS

In RRMS, the interplay between infiltrating pro-inflammatory $CD4^+$ T cells and CNS resident innate immune cells drives a pro-inflammatory response, which is amplified by the recruitment of peripheral lymphocytes. This includes the activation of $CD8^+$ T cells and autoantibodies-producing B cells. Together, the activated immune cells damage the myelin sheath and neuronal fibers in the CNS, as depicted in Figure 1 [3]. These processes can lead to the loss of neuronal and axonal function and scar formation, thereby impairing stimulus conduction [4].

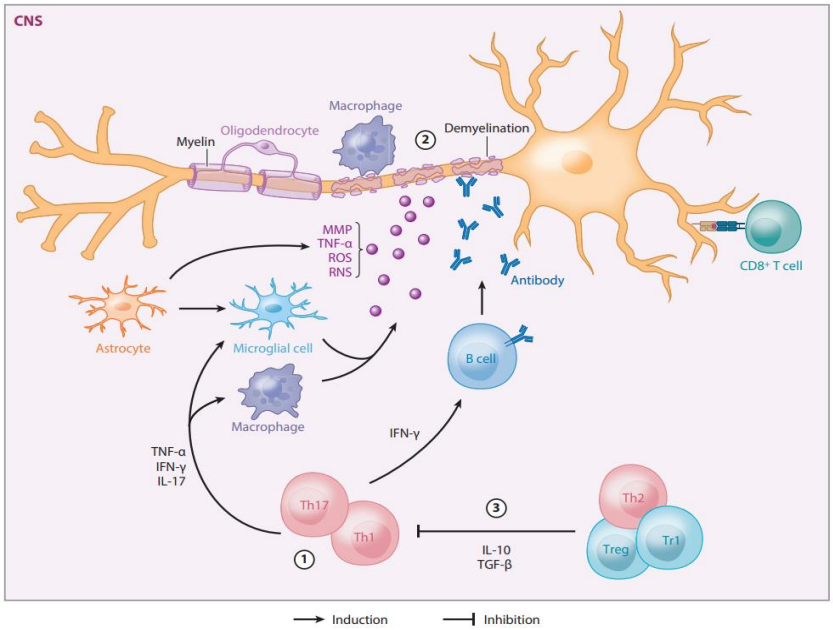

Figure 1. The immune response in RRMS. Immune cells involved in the autoimmune response against the myelin sheath of CNS neurons are shown in the figure. By producing cytokines, harming molecules and antibodies, the various immune subsets are involved in the breakdown of myelin and damaging neuronal fibers (Retrieved from Murúa et al., 2022) [3].

As aforementioned, patients with RRMS experience recovery after an exacerbation. This requires limitation of the immune response, to stop the destructive processes. Several regulatory immune cells have been identified for this purpose, both adaptive and innate. The suppressing function of these cells is mediated through various mechanisms, for instance through activation of the transcription factor FoxP3 or secretion of interleukin (IL) 10 [5].

In addition to the inhibition of the immune response, remyelination of the affected neurons is characteristic of RRMS. This process, mediated by the oligodendrocytes of the CNS, is only partial and leads to a myelin sheath thinner than the original. Remyelination does not succeed in progressive subtypes of MS [3].

1.1.2. PMS

Progressive manifestations of MS are characterized by severe neurodegeneration and demyelination in the cortex. As opposed to the frequent migration of lymphocytes over the BBB in RRMS, this happens only sporadically in PMS patients. The invaded lymphocytes aggregate in the CNS, mainly in the connective tissue areas. Subsequently, the cells are involved in the inflammation of the meninges and cortical demyelination. Whether the inflammatory aggregates contribute to the degeneration of the neural tissue or are activated upon this degeneration, remains unknown [5, 6, 7].

In addition to damaging immune responses in PMS, immune-independent mechanisms play an important role in disease progression. This includes damage that impairs the functioning of the neuron, such as oxidative stress due to iron release after demyelination. These immune-independent mechanisms amplify tissue damage, which can result in brain atrophy [5, 7].

1.2. Current therapies

A broad spectrum of so-called *disease-modifying therapies* (DMTs) is available for MS patients. In general, the currently available therapies focus on limiting the autoreactive potential of lymphocytes or stimulating regulatory immune cells. Hence, the drugs restrict the damage to neurons in the CNS [8].

When considering MS therapies, a distinction can be made between first-, second-, and third-line therapies, based on efficacy and severity of adverse events for the patient. Since there is only limited information about the usability of the third line, this category will not be discussed separately [9].

1.2.1. First-line therapies

Commonly used first-line therapies are interferon-β derivatives and glatiramer acetate. These therapies are safe and have high efficacy, mainly in treatment-naïve patients [8]. Interferon-β therapies have various effects, amongst which stimulating IL-10-producing regulatory T cells. The mechanism of action of glatiramer acetate is not fully understood but is thought to stimulate the expansion of various regulatory and anti-inflammatory cells [5]. In the case of highly active RRMS, cladribine can be used as a first-line therapy [10]. This drug specifically inhibits purine synthesis in lymphocytes, thereby inducing apoptosis and reducing lymphocyte number [5,10].

1.2.2. Second-line therapies

Second-line therapies are used in patients who experience disease activity despite the use of a first-line DMT. Natalizumab is such a therapy, regularly used in RRMS patients [8]. This antibody prevents the migration of lymphocytes over the BBB, by blocking adhesion molecules [5]. In addition to natalizumab, anti-CD52 and anti-CD20 monoclonal antibodies are used in the clinic. The mechanism of action of these therapies is focused on the reduction of lymphocyte function and numbers [10].

1.2.3. Therapies for PMS

The aforementioned therapies are shown to be effective in RRMS patients, but less in PMS patients. This can be explained by the dual importance of immune and immune-independent mechanisms in the pathogenesis of PMS [7]. Hence, modulatory therapies focusing on immune subsets could potentially not be sufficiently effective [5]. Currently, only two drugs are approved for the treatment of PMS patients, both having an anti-inflammatory effect [11]. Ocrelizumab is approved for the treatment of PPMS patients. This biological inhibits auto-reactive B cells by inhibiting the CD20 receptor. The drug Siponimod is approved for SPMS patients [10]. This drug inhibits the exit of lymphocytes from lymph nodes, thereby reducing the chance of an immune response in the CNS [5].

1.2.4. Characterization of MS therapies

It has to be noted that the recognition of MS therapies as first- or second-line can vary among patients and different drug authorities. It is possible that, based on a patient's disease activity, therapies defined as second-line are used as a first-line option. For instance, this is the case for the aforementioned natalizumab treatment. Furthermore, differences exist between countries when considering the indication of certain therapies as first- or second-line. An example is the biological alemtuzumab, used as a second-line therapy in Europe, whereas it is registered as a third-line therapy in the United States [9]. In this article, the focus will be put on the European perspective.

1.2.5. Treatment of MS exacerbations

The discussed therapies focus on managing the disease and limiting the number of exacerbations. Despite the use of DMTs, exacerbations, or relapses, can take place [5]. When this occurs, the corticosteroid methyl-prednisolone is frequently used to dampen the immune response in the acute phase. The drug does so through various mechanisms, among which are the downregulation of pro-inflammatory cytokine production and the induction of apoptosis immune cells. This leads to recovery from the relapse on a short-term [12].

1.3. Aim of the review

Although the currently approved MS therapies target the autoimmune response in various ways, even more strategies are required to be able to treat all patients effectively. In recent years, the autoreactive immune response and subsequent pathologic processes in MS patients have been studied closely. This has brought up new immunologic targets, but also the need to look beyond managing the immune response. For instance, the immune-independent mechanisms involved in disease progression in PMS require a different approach. Furthermore, new insights have been gained into how non-immunologic processes play a role in limiting disease progression. This allowed the generation of therapies aimed at ameliorating disease, through the recovery of imbalance, repair of injury, or restriction of the damage in the CNS. The current review aims to explore this new generation of therapies, by looking beyond conventional immune-modulating treatments.

2. Recovery

An emerging field of therapies focuses on the temporary abrogation of disease progression, by recovering imbalanced systems that drive disease progression. This chapter reviews two prominent therapies having such a recovering potential: autologous hematopoietic stem cell transplantation (aHSCT) and fecal microbiota transplantation (FMT).

2.1. Hematopoietic stem cell transplants

To halt the autoreactive immune response in MS patients, an attempt to create a balanced, tolerant immune system could be made. This goal can potentially be reached using stem cell transplantations (SCTs), of which the most promising is autologous hematopoietic stem cell transplantations (aHSCTs) [13]. The following paragraph gives an overview of the prospects of the application of this therapy in MS patients.

The effectivity of aHSCT in MS patients was shown in patients who underwent the procedure for another, most often oncological, reason and were coincidentally suffering from an autoimmune disease as well. It appeared that the transplantation led to the amelioration of MS symptoms caused by the autoreactive immune response [14]. In 1998, it was shown that remission was indeed induced using aHSCT in patients with autoimmune disorders, among which MS [14]. Many clinical trials have been performed since then, confirming the limitation of disease progression in many patients. However, the safety of the therapy varies amongst clinical trials, given the possible emergence of long-term neuroimmunological side effects not associated with the patient's original disease [15].

In short, the aHSCT procedure consists of stimulation of hematopoietic stem cells (HSCs), harvest, immune ablation, and engraftment of the harvested HSCs. Ideally, the transplanted stem cells initiate the generation of new, non-autoreactive immune cells [13].

2.1.1. Effectivity of aHSCT in RRMS and PMS patients

Over the years, it has been studied regularly which patients can be treated successfully with aHSCT [15]. A longitudinal study by Boffa et al. (2021) has shown that aHSCT was successfully halting disease progression in 70% of the included RRMS patients for 10 years post-aHSCT. This was also the case in around half of the included PMS patients. Based on the absence of progression in the majority of patients, the authors concluded that aHSCT is beneficial for both RRMS and PMS patients, who experienced disease progression before the treatment [16]. These results were in accordance with many others, showing stabilization or decrease of disease activity [15]. In other studies, it was shown that the mortality risk is higher for severely disabled patients, due to a high risk of complications during the procedure. This led to the recommendation to exclude these patients from aHSCT [17]. Combining the results of many studies, the current, consensus about aHSCT in MS patients is that it should be considered a potentially useful treatment for young patients, in whom DMTs were shown to be ineffective and who have a short disease duration, but rapid disease progression. This includes both RRMS and PMS patients [17].

2.1.2. Immune ablation or immune suppression

As mentioned before, the procedure of aHSCT includes the ablation of the existing immune cells. In the first studies, this was done using the high-intensity chemotherapeutic agent busulphan. However, this regimen is associated with increased mortality [13]. Since low-intensity treatments have a higher chance of renewed disease activity, the intermediate intensity was proposed as being optimal. The most frequently used intermediate method nowadays is 'BEAM', a mixture of several chemotherapeutic agents, combined with ATG, a T cell-depleting globulin. Together, the compounds remove the majority of red and white blood cells, after which transplantation can be initiated [18].

2.1.3. Immunologic processes behind aHSCT effectivity

The main goal of aHSCT is to remove autoreactive lymphocytes and to rebuild a self-tolerant immune system by engraftment of HSCs. As mentioned before, several immune subsets play a role in the pathogenesis, of which lymphocytes are the most frequently studied, also in the context of aHSCT.

2.1.3.1. Re-emergence of lymphocytes

In the period after engraftment, the B and T cells reappear, although at various time points post-transplantation. B cell populations on the one hand appear within 6 months, whereas the number of newly generated T cells increases only after 6 months [19]. In the case of B cells, the restored population mainly consists of plasma cells, probably generated from memory B cells that survived the treatment. Newly formed memory B cells appear to be formed 3 months after transplantation, but the levels take at least a year to fully recover [20].

The restoration of T cell populations post-aHSCT follows the principle of 'lymphopenia induced proliferation': stimulation of rapid expansion of the $CD8^+$ T cell population, resulting in more $CD8^+$ cells than $CD4^+$ cells. After 12 months, the $CD4^+$ T cell population increases by the egress of these cells from thymic tissue. Although the size of the thymus declines with age, it has been shown that the remaining tissue can stimulate its growth itself after drastic lymphocyte-depleting therapies such as aHSCT. This process is also known as 'thymic rebound' [21]. Muraro et al. (2014) showed that the early increase of $CD8^+$ cells is due to the expansion of surviving effector cells. The relatively late $CD4^+$ T cell expansion on the other hand is due to the development of a new population, containing T cell receptors (TCRs) that were not detected pre-treatment. Interestingly, it was shown that the diversity of the TCR pool is of high importance since a lack of variation was associated with the failure of the treatment [22]. This indicates that a highly diverse TCR repertoire is needed for establishing a normal, tolerant immune system.

By studying the various $CD4^+$ subsets post-aHSCT, Darlington et al. showed that the activity of Th17 cells is drastically reduced after aHSCT, whereas the activity of Th1 and Th2 cells is not. Given the great involvement of Th17 cells in the pathogenesis of MS, these results could serve as an explanation for disease attenuation post-transplantation [23].

2.1.3.2. Upregulation of inhibitory receptors post-transplantation

In 2016, Arruda et al. studied the immunological profile of patients who experienced either short- or long-term amelioration of disease activity after aHSCT [24]. The results of this study indicated that patients in whom the therapy had been successful, had a larger population of *programmed cell death-1 protein* (PD-1) positive $CD8^+$ T cells and PD-1-positive $CD19^+$ B cells [24]. The activation of this protein is associated with the suppression of the according immune cells. The counts of these PD-1-positive lymphocytes were lower in patients who experienced disease progression after aHSCT. Taking the results of various studies together, Arruda et al. hypothesized that PD-1 is involved in inhibiting the expansion of autoreactive $CD8^+$ cells during immune reconstitution after aHSCT [24]. Based on these results, it can be hypothesized that the downregulation of PD-1 on $CD8^+$ cells plays a role in the prolongation of the autoimmune response in active MS. Further studies on this topic should determine whether this is indeed the case, how PD-1 prevents autoimmunity in a normal situation and whether this can be targeted therapeutically.

2.1.4. Immune tolerance

Ideally, patients develop immune tolerance after the reconstitution of the immune system. The absence of disease progression after aHSCT suggests the existence of tolerant or suppressive immune cells. How regulatory T cells play a role in this process, remains unknown, since changes in the relative numbers of these cells post-aHSCT differ among studies [25]. Studies on the effect of aHSCT on regulatory

cells in other autoimmune diseases have shown that more diverse and effective cells are present after transplantation. Whether this is the case in MS patients who underwent aHSCT, remains unknown [21].

2.1.5. Innate immune cells in aHSCT

Information about the recovery of innate immune cells remains after aHSCT remains limited. Only a few studies describe the dynamics of Natural Killer (NK) cells after the treatment. The importance of these cells became clear from studies showing the worsening of autoimmune disease after the depletion of these cells [26]. This was underpinned by the study of Darlington et al. (2018), which showed that NK cells recover rapidly post-aHSCT. The researchers hypothesized that the rapid emergence of these cells could be due to the outgrowth of remaining NK cells, which survived immune ablation. Interestingly, the researchers also showed that the numbers of NK cells were inversely related to the numbers of Th17 cells: a high level of NK cells was accompanied by low levels of Th17 cells [26]. This indicates that the innate immune system plays an important role in preventing the re-emergence of an autoimmune response post-aHSCT. In addition, Ruder et al. (2021) reported the dynamics of NK cells and innate-like T cells. It was once again shown that NK cells with regulatory capacities are increased after aHSCT, whereas the number of pro-inflammatory NK cells decreased [27]. The relative and absolute increase of regulating NK cells were underpinned in a subsequent study by Visweswaran et al. (2022) [25]. Moreover, the results of Ruder et al. indicated a decrease of innate-like T cells, among which γδT cells, which have pro-inflammatory properties [27]. These results underline the importance of altered innate immune cell dynamics post-aHSCT.

In short, the results of the studies shine a new light on the involvement of innate immune cells in the pathogenesis of MS and inhibition of disease progression after aHSCT. More research should be performed to confirm these results and to determine to what extent the innate and adaptive responses play a role in disease amelioration after aHSCT.

2.1.6. AHSCT versus DMTs

A question that might come up at this point is whether aHSCT is more effective than treatment with approved DMTs. An international Phase II clinical trial by Burt et al. (2019) addressed this question, by assessing the progression of disease in RRMS patients who were either treated with aHSCT or a DMT of high efficacy. Although the group size and follow-up time were limited, it was shown that patients treated with DMTs experienced disease more frequently than patients who underwent aHSCT [28]. A similar result was shown for alemtuzumab, the strongest DMT available. Boffa et al. found that aHSCT was more potent to prevent disease progression in patients with aggressive RRMS, as compared to alemtuzumab [29]. The results of these studies suggest that aHSCT is more efficiently inhibiting disease progression than highly efficient DMTs. In both studies, this effect is apparent for several years post-treatment [28, 29].

Several points need to be discussed when considering the results of the aforementioned studies. Firstly, Burt et al. randomly assigned half of the patients to the DMT group, after which treatment with a higher efficacious DMT was initiated. The exact DMT which was going to be used was decided upon their neurologist's advice, which diminishes the chance of prescribing an ineffective DMT. However, it could still be possible that the selected DMT is not functional for a patient. Although it is complicated to exclude this problem without a period of monitoring the effectivity of the DMT before the research, it can be minimized by including more patients per subgroup. Furthermore, only RRMS patients were included in the cited studies. It can be useful to include PMS patients in these studies as well, given the potential effectivity of aHSCT in these patients as discussed in paragraph 2.1.3.. Only a few therapies are currently available for these patients, which are described as being highly effective. It would therefore be interesting to determine whether the effectiveness of these therapies outcompete aHSCT.

Amoriello et al. (2020) specifically compared the *T cell receptor* (TCR) diversity among patients who received either natalizumab or aHSCT. It was shown that the naïve $CD8^+$ population was more diverse after aHSCT as compared to natalizumab treatment. This indicates that the immune system after aHSCT can mediate better protection against a wide variety of antigens [30]. It should be noted however that natalizumab is an immunosuppressant, thus impairing the general immune response. A low diversity of TCRs will therefore not be the main reason for decreased protection from antigens in patients using natalizumab.

In the same study, it was shown that the percentages of memory T cells, both $CD4^+$ and $CD8^+$, appeared to be higher in patients who were treated with natalizumab. These clones were shown to be persistent during the entire duration of the study, 24 months. [30]. This implies that memory, including memory of the autoreactive immune response, is retained using natalizumab. The low numbers of memory lymphocytes after aHSCT imply that this therapy is more efficiently wiping out the autoreactive immune response. Whether the decreased percentages of memory T cells up until 2 years after aHSCT is specific to MS patients, is not known. A future study comparing MS patients and patients treated for another reason could provide an answer to this question.

2.1.7. Ongoing clinical trials on aHSCT

Despite many clinical trials showing a halted disease progression in many patients, concerns prevent approval of the therapy in many countries. Ongoing debates are, among others, about the selection criteria of patients and the intensity of the treatment regimen [17].

To determine whether aHSCT can be safely implemented in the clinic, several clinical trials are ongoing. In Scandinavia and The Netherlands, a multicenter, Phase III clinical trial is ongoing (NCT03477500). This study is comparing non-ablative aHSCT and several strong immunomodulatory treatments in RRMS patients who experience a high disease activity. Instead of immune ablation using BEAM, a low concentration of the chemotherapeutic agent cyclophosphamide and ATG is used to mildly suppress the immune system. The generation of stem cells in the bone marrow is stimulated using a granulocyte colony-stimulating factor. According to the authors, the results of this study could serve as a basis for future decisions on the approval of aHSCT as a treatment for MS patients [31].

2.2. Fecal microbiota transplantation to restore disbalance

Over the last decade, the importance of gut microbiota in health and disease became more apparent. In healthy individuals, the composition of this microbial community is highly diverse and contains mainly symbionts, contributing to overall health [32]. Deregulation of the composition of this microbial community, or dysbiosis, can result in the colonization of pathogenic strains, which has health consequences. Dysbiosis of the gut microbiota has also been detected in MS patients. Nonetheless, it remains unknown whether this altered composition is a cause or consequence of disease, indicating the need for additional research on this topic [33].

2.2.1. The interplay between the intestinal microbiota and the immune system

In light of autoimmune diseases, the interaction between gut microbiota and the immune system needs to be discussed. It has been shown in various ways that intestinal microbes have direct contact with the lymphoid tissue in the gut. Under normal circumstances, the bacteria promote the function of regulatory lymphocytes, to prevent inflammation. In case of damage, the microbiota induce an inflammatory response, mainly mediated through macrophages and Th17 cells [33]. Given the fact that the influence of the gut microbiota is not limited to the gastrointestinal tract itself, a link was drawn between systemic immunity and the composition of the intestinal microbes [33]. As such, studies showed a link between the enrichment of certain microbiota in patients and the deregulation of the immune

response [34]. For example, more IL-17-promoting bacteria were found in the microbiota of rheumatoid arthritis patients. Since this interleukin is of importance in the pathogenesis of the disease, this finding might imply the involvement of the microbiota in exacerbations [34]. In MS specifically, only small differences between the composition in patients compared to healthy controls were shown in multiple studies [35]. However, a recent study from the International Multiple Sclerosis Microbiome Study showed a significant increase in pro-inflammatory bacterial strains and a decrease in anti-inflammatory strains. Furthermore, regular compositional changes in patients treated with DMTs were detected. The implications of these differences remain unknown and leave room for further studies on the link between MS and the intestinal microbiota [36]. Further research is also needed to show the effect of the enriched microbiota on the local and systemic immune system, to clarify whether this impacts the autoreactive immune response.

2.2.2. Fecal microbiota transplantations in MS

The rationale of fecal microbiota transplantations (FMTs) is to correct dysbiosis, by infusing the balanced microbiota of a healthy donor using fecal material. In theory, the infused microbes can settle and create a balanced community in the colon, restoring, among others, immune homeostasis [37]. One study has been published that describes the application of FMT in RRMS patients, which resulted in partial engraftment of donor microbiota [38]. Some of the strains that were enriched post-FMT have a known anti-inflammatory function, thus hypothetically mitigating the autoreactive immune response. However, a reduction of the concentration of several pro-inflammatory cytokines could not be shown. Lastly, the production of Vitamin K, which is beneficial to MS patients, was shown to be increased after FMT. It could thus be concluded that FMT can potentially be useful for MS patients in reducing inflammatory activity [38]. Since FMT as a treatment for MS patients is still in its infancy, it is not known yet whether it can affect disease progression or symptoms. Once the effectivity of FMT is established, clinical effects can be assessed in additional studies.

2.3. Concluding remarks

In this chapter, the mechanism behind aHSCT and FMT and their applicability were discussed. AHSCT is a promising therapy for MS patients, given the chance of a successful stop of the disease. As shown by Boffa et al. (2021), the absence of disease progression can last at least for 10 years [16]. However, it remains unsure whether this is retained for an even longer period. Given the importance of either genetic, environmental, or infectious components, it can be hypothesized that there is a chance of the re-development of MS. This could be taken into account when determining the best treatment option for patients, given the high costs of the therapy. However, it can be debated whether it is ethically justifiable to deny patients such a life-changing therapy because of uncertainties about the effectiveness of the therapy over multiple decades.

Concerning FMT, it has to be noted however that the intestinal microbiota have a high inter-individual variability, which complicates the standardization of this therapy. Research data should therefore be translated to individual patients: personalization of the therapy would be required, based on personal needs of enrichment or reduction of certain bacterial strains. It can therefore be doubted whether this therapy has the potential to be applicated on a large scale.

3. Repair and restriction of neuronal damage

Currently, there are no therapies available that repair the damage following the autoimmune response. Such therapies are being developed, but are still in their infancy [39]. This chapter provides an overview of several promising repair-inducing therapies for MS patients.

3.1. Remyelination

The myelin sheath is the protective layer wrapping the fragile neurons in the CNS. In addition to its protective function, it plays a crucial role in accelerating signal conduction. When damage is done to the myelin sheath, oligodendrocytes in the CNS produce new myelin to repair the affected areas. Since this process of remyelination does not always successful in MS patients, it was questioned whether it can be therapeutically induced. Indeed, several compounds have been identified as potential remyelination-promoting agents. These compounds either directly promote oligodendrocyte function or indirectly stimulate remyelination by suppression of inhibitory signals [40].

3.1.1. Direct remyelination

Clemastine, also known as clemastine fumarate, is an FDA-approved anti-histamine drug having remyelinating capacities. The compound does so by stimulating the differentiation of oligodendrocyte progenitor cells (OPCs), leading to functional oligodendrocytes [41]. In a phase II clinical trial in 2017, MS patients who had suffered from optic neuritis were treated with the drug. It was shown that after treatment with clemastine, the speed of signal conduction in the optic nerve was increased compared to the situation before the treatment. This indicates a re-established myelin layer because of the signal-accelerating function of myelin. Whether this effect is sustained in the long term fell outside the scope of this study. Furthermore, the authors noted that it remains to be studied whether clemastine can induce clinical improvement since this study failed to generate unambiguous results. It was concluded that additional research is required to determine the potential of clemastine in repairing myelin damage [41].

In addition to clemastine, the drug metformin was shown to have a promising remyelinating potential. This drug, approved as a therapy for diabetes mellitus Type II, was shown to stimulate the generation of oligodendrocytes in various murine demyelination models [42]. Based on the results of the studies on clemastine and metformin in the context of remyelination, the applicability of combination therapy is being studied in a Phase II clinical trial (NCT05131828). The study includes RRMS patients who suffered from optic neuritis, thus showing a decreased signal conduction in the optic nerve. Again, measurement of the speed of this conduction after the use of the combination therapy will indicate whether remyelination has occurred [43]. If the combination therapy indeed increases conduction speed, further studies can be initiated to determine whether it also improves clinical symptoms. It has to be noted however that it is not possible to conclude whether remyelination has occurred in human models. Only an indication can be obtained by measuring signal conduction.

3.1.2. Indirect remyelination

Another approach to induce remyelination is to suppress inhibitory signals that prevent the initiation of myelin repair. Opicinumab is such a drug, specifically promoting oligodendrocyte differentiation by blocking the neuronal receptor LINGO-1. Given its mechanism of action, it was hypothesized that opicinumab leads to remyelination [44]. In a phase II clinical trial, the effect of the therapy was studied in RRMS patients. However, it was shown that opicinumab did not evoke a substantial clinical improvement in the patients receiving the therapy as compared to the placebo group [44]. Although the authors hypothesized that certain subgroups of patients could still benefit from the treatment, the studies on opicinumab were discontinued after phase II due to disappointing results of clinical trials [40, 44].

3.1.3. Remyelination in PMS

One could question whether induction of remyelination is possible in PMS patients because of severe demyelination. This implies dysfunction of oligodendrocytes or the precursors of these cells. Nonetheless, lesions that oligodendrocytes are sufficiently present in not remyelinated lesions [45]. Consequently, it has been hypothesized that agents activating these OPCs could initiate remyelination. However, not all patients appear to have oligodendrocyte pools in lesions. Furthermore, it was noted that the remyelinating capacity of oligodendrocytes declines with age. Hence, therapies inducing remyelination will plausibly not be effective in all MS patients [45]. If remyelination-inducing therapies become available, it is thus of high importance to study the inclusion and exclusion criteria of patients thoroughly.

3.2 Neuronal repair and restriction of neuronal injury

An important hallmark of MS in both early and late stages is neuronal injury and subsequent degeneration of neurons and axons [46]. These processes lead to severe clinical symptoms, such as increasing immobility [4]. In theory, neuroprotective therapies can, as the name implies, prevent degeneration and its accompanying symptoms [46]. This paragraph discusses several neuroprotective therapies that are currently being studied.

3.2.1. The potential of mesenchymal stem cells in MS patients

As discussed in the previous chapter, the therapeutic potential of SCTs is being studied for MS. Mesenchymal stem cells (MSCs) are potentially interesting due to their wide variety of functions, among which neuroprotection. In a mouse model of MS, the transplantation of autologous MSCs led to the reduction of autoreactive $CD4^+$ cells and the amelioration of clinical symptoms [13]. In 2021, Uccelli et al. reported the results of a Phase II trial on transplanting MSCs in RRMS and PMS patients intravenously. It was shown that the therapy was safe, but displayed neither clinical effects nor neuroprotective effects. Although the same results were generated by several other small-sized clinical trials, others identified a significant disease-ameliorating effect of MSC transplantation. Uccelli et al. subsequently discussed that the differences between these studies are probably due to varying and suboptimal treatment protocols, for instance by including MS patients in different stages of the disease. Furthermore, it was noted that the route of administration could have an impact on the outcome of the study: intrathecal administration could be preferred over the intravenous route [47]. Future research should thus keep an eye on specifying the included patient group and standardizing the procedure. This would give a better insight into the effectiveness of MSCs in the treatment of MS patients.

3.2.2. Small molecules for neuronal protection

In addition to MSCs, several small molecules have been identified as protective agents of neuronal tissue. The drug ibudilast inhibits phosphodiesterases, thereby preventing neuronal and oligodendrocyte death [46]. Furthermore, the drug mediates neuronal protection by preventing immune-mediated inflammation, for instance by reducing pro-inflammatory cytokines [11]. Clinical trials have shown that varying dosages of ibudilast reduce disease progression and neural damage in both RRMS and PMS patients [11,46]. Although these results are promising, further research has to be performed to pinpoint how safe the drug is and which dosage is most optimal.

Statins are well-known as cholesterol-lowering drugs in cardiovascular diseases but have been found to have additional neuroprotective effects. For example, statins prevent nitric oxide production, thereby limiting the generation of a neurotoxic environment. Furthermore, the generation of new neurons and synapses can potentially be stimulated by statins through processes that are not fully understood. Specific statins, among which simvastatin, can cross the BBB, thus acting directly in the

CNS [48]. Trials in several animal models have shown that simvastatin specifically reduced lesion formation. A subsequent Phase II trial indicated reduced CNS damage and clinical symptoms in SPMS patients treated with simvastatin, as compared to a placebo-treated group [11]. A subsequent trial (NCT03896217) is currently running, to determine whether the disease-ameliorating effects of simvastatin are due to alteration of brain perfusion in SPMS patients [49].

3.3. Concluding remarks

The previous paragraphs give an overview of only a part of the therapies being developed for repairing or preventing the damage following the autoreactive immune response in MS patients. It is promising that certain approved drugs are being investigated for their remyelinating and neuroprotective capacities. If these therapies are eventually shown to be effective, approval for their use in MS patients can be accelerated.

Other therapies, ranging from ion channel blockers to approved MS therapies, have been shown to have a neuroprotective potential in MS patients as well. However, some compounds did not show a consistent reduction of disease activity, and their development was terminated. An overview of therapies that are still being developed is given in Table 1. The clinical trials that are still running, need to conclude whether repair and restriction can become additional goals of MS treatment.

Table 1. Compounds that are studied in clinical trials for their neuroprotective or remyelinating capacity.

Compound/Therapy	Proposed effect	Phase of trial
ATA188	Neuroprotection through the elimination of B cells infected with Epstein Barr Virus, which could play a role in the autoreactive response in MS [50].	Phase II trial studying the effectiveness of the therapy in PMS patients [50].
Alpha-lipoic acid	Neuroprotection through reduction of T cell migration, pro-inflammatory cytokines, and oxidative stress. Previous studies showed decreased brain atrophy in PMS patients treated with the compound [11].	Phase II trial in PMS patients [11].
Bazedoxifene Acetate (estrogen receptor modulator)	Induction of remyelination through stimulation of oligodendrocyte maturation [51].	Phase II in RRMS patients [51].

The consulted literature on remyelination did not provide information or a hypothesis on the quality of the generated myelin. This would be of interest since repaired myelin in MS patients is often thinner than the original layer. In line with this, it can be hypothesized that remyelinating therapies also result in a suboptimal myelin layer. If a normal, healthy myelin layer is generated after treatment, it would be reasonable to apply the therapy from an early phase on. This would prevent the generation of insufficient, thin myelin layers. Further research should thus be conducted on whether it is possible to get an indication of the thickness of the myelin.

4. Other considerations

As mentioned in the introduction, it is not yet fully understood what drives MS pathogenesis. Over the last few years, much attention has been put on the influence of lifestyle on the disease course and disease progression in MS patients. This chapter delves deeper into the impact of nutrition and physical activity on MS.

4.1. Nutrition

The correlation between the immune response and nutrition has gained attention in the past years. A Western diet for instance is associated with a higher degree of inflammation. Such a diet consists of processed, high-fat, high-sugar, and high-salt foods that lack, among other things, fibers and vitamins. The diet is associated with a higher level of inflammation, thus a more active immune system. This higher activity can affect all tissues because metabolic processes and systemic and tissue-specific immune responses influence each other. Specifically, the nutrient cholesterol is associated with the activation of inflammasomes, thereby inducing a pro-inflammatory response. How this process exactly contributes to the state of higher inflammation, is not yet fully understood [52].

4.1.1. Western diet and the intestinal microbiota

The intestinal microbiota are associated with the pro-inflammatory state when adhering to a Western diet. As discussed in paragraph 2.2, the intestinal microbiota play a role in the pathogenesis of MS. Since the composition of this microbial community is affected by nutrition, it is not unexpected that diet can interfere with the immune response, thereby contributing to the pathogenesis of MS [53]. In a normal situation, the microbiota contribute to immune homeostasis in the intestines through multiple complex processes. This includes the stimulation of the production of mucosal immunoglobulin A, which serves as the first line of defense against pathogens. If the composition of the microbiota becomes dysbiotic, the regulation of the immune system is impaired as well. Generally, this situation leads to a thinner mucus layer, less mucosal immunoglobulin A, and a higher susceptibility to infections. Furthermore, dysbiosis of the intestinal microbiota is associated with systemic inflammation [52]. Hence, the pro-inflammatory profile of the microbiota in MS patients following a Western diet promotes an undesirable systemic immune response, which could favor the autoreactive response [54].

4.1.2. Anti-inflammatory nutrition

The anti-inflammatory role of certain nutrients is potentially interesting in the treatment of MS patients. A frequently described diet that reduces the activity of the immune system is the Mediterranean diet. This diet is characterized by the replacement of saturated fatty acids with unsaturated fatty acids and the consumption of fiber through vegetables and fruits [54]. Unsaturated fatty acids are associated with having an anti-inflammatory function by inhibition of inflammasomes and pro-inflammatory cytokines and the stimulation of a regulatory response [52]. In RRMS patients, the intake of the poly-unsaturated fat omega-3 led to the amelioration of disease-associated symptoms and the levels of pro-inflammatory markers [54]. Other studies showed a comparable effect using other unsaturated fatty acids, such as omega-6 [53]. Hence, it can be concluded that unsaturated fatty acids can ameliorate immune activity in MS patients, although definitive advice on which to use remains absent.

Fibers, on the other hand, have an anti-inflammatory role through direct interaction with the microbiota. Through the intake of fibers, certain microbiota are stimulated to produce *short-chain fatty acids*, that counteract inflammations [52]. The disease-limiting capacity of dietary fibers has been shown to reduce fatigue and increase physical ability in MS patients [54].

In addition to the discussed macronutrients, micronutrients have been shown to have disease-limiting potential as well. An overview of some compounds belonging to this group is shown in Table 2.

Table 2. Micronutrients that could beneficially affect the disease course of MS patients.

Compound	Mechanism of action	Effect on MS patients
Vitamin D3	Inhibits the differentiation of Th1 and Th17 cells and increases the number of regulatory T cells in an MS animal model. A low concentration of vitamin D in the blood is associated with an increased risk of developing MS [54].	Amelioration of MS symptoms and reduction of lesions in the brain. Some studies could not show a beneficial effect [54].
Polyphenols: flavonoid (e.g. in green tea, vegetables, and fruits)	Various mechanisms are shown in animal models, among which are the reduction of oxidative stress and the decrease of pro-inflammatory cytokines [55]. Specifically, epigallocatechin gallate-3, a component of green tea, contributes to neuroprotection in animal models of MS [46].	Reduction of markers of inflammation as well as a moderate clinical improvement. Curcumin, a component of turmeric, reduced the activity of pro-inflammatory processes. Furthermore, this flavonoid can restore the integrity of the BBB [54].
Polyphenols: nonflavonoid (e.g. in berries and peanuts)	Reduces pro-inflammatory molecules and cells and promotes the integrity of the BBB in an animal model of MS. Resveratrol, for example, reduced the degradation of tight junctions in the BBB [54].	Decrease of matrix metalloproteinase 9, which is involved in the disruption of the BBB in MS patients. Alteration of clinical symptoms could not be shown [54].

4.2. Physical activity

It has been shown that physical activity (PA) and exercise have multiple effects on the CNS and the immune system that could potentially contribute to disease amelioration in MS patients [56]. It became clear from multiple studies that this can be mediated through various processes. This paragraph discusses some of the potential processes, either detected in animal models or MS patients after PA or exercise. This chapter uses 'PA' for the movements accompanying day-to-day tasks and 'exercise' as the repeated movement with a specific objective (e.g. gaining muscle strength) [56].

4.2.1. Upregulation of brain-derived neurotrophic factor in MS patients

Brain-derived neurotrophic factor (BDNF), produced by microglia and neurons in the CNS, is an important molecule in the CNS due to its neuroprotective and -regenerative potential. In MS patients specifically, it was shown that this factor is highly present in demyelinated areas of the CNS, indicating its important function in the restoration of the neural tissue [57]. Interestingly, it has been shown that the blood serum concentration of BDNF in the periphery is increased upon exercise [58]. Given its potential clinical benefit, studies have been performed to study the upregulation of BDNF in MS

patients after exercise. Indeed, an increased blood serum concentration of this factor could be detected in RRMS patients [57]. Whether the same effect would occur in PMS patients, was studied by Briken et al. (2016). The group concluded that the concentration of BDNF in the blood serum increased after only a short period of exercise, but decreased quickly after the training stopped. This could potentially be due to the relocation of the factor from the blood to the CNS. Interestingly, the authors also reported a potential correlation between exercise intensity and BDNF production: the levels of BDNF were higher in patients who did a more intense exercise [58]. Whether exercise intensity indeed plays a role in the production of the compound and is the reason behind the absence of BDNF in some patients after exercise, should be shown in follow-up studies.

4.2.2. Structural changes in the brain

Several studies have been performed to study the effect of PA on the integrity of neural tissue. Studies have shown that exercise can lead to structural changes in various areas of the CNS, whereas PA did not result in significant changes [59]. It was shown that exercise did not only lead to the restoration of the integrity of white and gray matter but also normalized or increased the size of specific areas in the brain. In some studies, structural changes or restorations were associated with the improvement of cognitive functions [59]. It has been questioned whether these improvements after exercise could be associated with BDNF. Indeed, several studies show the simultaneous rise of BDNF concentration and the improvement of cognitive impairments. It must be noted however that most studies included only a small number of patients, most of them being diagnosed with the RRMS subtype [57]. Furthermore, some studies failed to detect any structural changes to the tissue [56]. To determine the exact effect of exercise on brain structure in MS patients and to determine the applicability of PA in the clinic, further studies have to be performed. Moreover, patients being diagnosed with different subtypes of MS should be included in the studies, to determine whether structural alteration can still occur in progressive variants of the disease.

4.3. Clinical trials on lifestyle in MS

In the Netherlands, a large clinical trial has been initiated to study the effect of lifestyle on disease activity in RRMS and PMS patients. During a period of two years, the participants follow an online program that focuses on nutrition, physical activity, relaxation, and sleep. Using questionnaires, the participants' disease course will be assessed at various time points. The study includes adult patients with either RRMS or PMS [60]. The voluntary participation of patients implies that the studied group consists of highly motivated individuals, willing to adapt their lifestyle according to the information they receive.

4.4. Concluding remarks

In conclusion, nutrition and PA can have anti-inflammatory effects and thereby contribute to the amelioration of MS symptoms. This is mediated through various processes, among which are the reduction of pro-inflammatory cytokines and the production of the neuroprotective factor BDNF. When considering the integration of lifestyle adaptation in the clinic, the feasibility should be critically assessed. An important aspect of this adaptation is the willingness to make changes in the personal life. Assuming that the voluntarily enrolled participants in the studies are highly motivated to adapt their lifestyle, it can be doubted whether the data can be translated to patients in the clinic. This will probably lead to lifestyle adaptation remaining advice, rather than treatment.

Regarding nutrition specifically, it should be noted that MS therapies have a composition-altering effect on the gut microbiota [36]. Since great differences exist between the various therapies, it can be assumed that each alters the intestinal microbial community differently. This could hypothetically lead to nutrients having a different effect on a patient's health than described in the cited literature. As

such, studies on the effect of nutrition on MS disease course should consider participants' therapy as a potential confounder. This would make study results better translatable to targeted nutritional advice.

Lastly, it is doubted whether PA can ameliorate symptoms in all stages of MS. In most clinical trials on this topic, patients who had been diagnosed with the disease for multiple years were included. Since the repairing function of PA would be useful from an early stage of the disease, it can be hypothesized that the effect will be most optimal in these patients [56]. On the other hand, the partial or full immobility of PMS patients generally results in less PA. Hypothetically, regular and low-level PA might therefore have a relatively great effect in these patients, as compared to mobile RRMS patients. To determine whether this hypothesis is correct, additional studies on the effect of PA on patients in various stages of the disease are required.

5. Conclusion and future perspectives

Over the last few years, many studies have been performed to study and develop new therapies for MS patients. Since the current standard immune modulatory therapies are not effectively inhibiting disease progression in all cases, there is a need for this new generation of therapies. By looking beyond the autoreactive immune response as the main target of therapy, new strategies have been developed. In this review, an overview is provided of these innovative, possible MS treatments, which have the potential to be integrated into the standard treatment of MS patients.

Whether the discussed therapies are suitable for treating MS patients, is still unsure. To create certainty about applicability, further studies are required. Firstly, many of the cited studies show a positive effect of the therapy in a small group of patients. Furthermore, only a limited number of longitudinal studies are available. Hence, larger studies, including more patients and prolonging over a longer period, should be performed to confirm the results of previous, small studies. However, it should be noted that the initiation of large studies depends on earlier results and the safety of the treatment.

Moreover, details about the optimal time to initiate treatment and the targeted patient group remain to be identified. For instance, it can be hypothesized that remyelination will be most effective in the first stage of the disease, but is needed to a greater extent in PMS patients. The same accounts for therapies inducing neuronal protection. Future studies should therefore attempt to determine the most optimal timing of therapy initiation.

Lastly, most of the discussed therapies will plausibly remain supplementary to the immune modulatory therapies. Hence, it is important that both therapies do not interfere with each other and function properly. If a new therapy is undoubtedly shown to be effective for MS patients, a new phase of research should be initiated in which combinations of conventional and new treatments are studied. Since the current immune modulatory drugs differ greatly from each other, it can be hypothesized that differences will exist between the effectiveness of certain combination therapies. Consequently, the results of these studies will enable the most optimal usage of the new generation of therapies.

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