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Immunosuppression in relapsing remitting multiple sclerosis: moving towards personalized treatment

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Abstract

Introduction

Therapeutic armamentarium in Multiple Sclerosis (MS) has radically changed in the last few decades due to the development of disease modifying treatments (DMTs) with highly selective mechanisms of action.

Areas covered

In this review, the authors will focus on the current role of immunosuppressive DMTs in the management of the relapsing remitting form of MS (RRMS), moving from the rationale of its use and looking at the possibility to design an idealistic scenario of a personalized approach for each single patient.

Expert opinion

Questions remain open about whether initial high-efficacy immunosuppressive DMTs improve long-term outcomes, whether prolonged exposure to these agents increases adverse events and what the strongest early surrogate markers are for predicting long-term treatment responses to high-efficacy drugs. In this way, the immunosuppressive DMTs, are used to hit the immune system early and hard with the idealistic goal of striking the autoimmune activities before the neurological damage becomes irreversible.

Keywords: immunosuppression, personalized therapy, multiple sclerosis, induction, therapeutic approach

Article highlights:

- A challenge encountered by Multiple Sclerosis specialists is represented by the therapeutic management of patients with aggressive forms of Multiple Sclerosis or of patients who are intolerant, non-responsive to approved disease modifying treatments.

- Inflammation is the unique target of our available immune therapy and any realistic therapeutic strategy should be based on impacting the neuroinflammation with a range of possibilities.

- Questions remain open about whether initial high-efficacy disease modifying treatments improve long-term outcomes, whether prolonged exposure to these agents increases adverse events and what the strongest early surrogate markers are for predicting long-term treatment responses to high-efficacy drugs.

1. Introduction

Multiple Sclerosis (MS) is an immune-mediated neurodegenerative disease with a complex etiopathogenesis in which both genetic and environmental factors interact (1). MS affects an estimated 2.5 million people worldwide, with a higher prevalence and incidence in the northern hemisphere (2, 3). The clinical course of MS is unpredictable, and its management may require life-long pharmacological and non-pharmacological interventions, and the choice of the disease modifying treatments (DMTs) represents a crucial moment in MS management (1).

The most used therapeutic approach is represented by maintaining the patient on the same DMT until it no longer shows efficacy, tolerability and safety, or until it is deemed no longer necessary to continue (4). If any of these goals is not reached, it can be considered a therapeutic switch (4, 5). Advances in understanding of the disease mechanisms and the dynamic nature of the disease have brought around 12 DMTs to market in many countries. A challenge encountered by MS specialists is represented by the therapeutic management of patients with aggressive forms of MS or of those who are intolerant, non-responsive, or refuse to receive the current Food and Drug Administration (FDA) and European Medicines Agency-approved (EMA) DMTs (6). Growing evidence suggests that starting

DMT as soon the diagnosis is reached should modify the disease course, introducing the concept of “therapeutic window” (4, 7).

In the current clinical practice, the most important therapeutic decisions, which are represented by the start and the change of DMTs, are based almost exclusively on the data of MS disease activity: a) frequency, severity, and rate recovery from relapses, b) the degree of neurological impairment assessed by several scales, c) the “lesion burden” and in particular the presence of active enhancing lesions at brain and spinal cord magnetic resonance imaging (MRI) (8). In the last years, great attention has been posed on the presence of concomitant medical illnesses, the use of other medications, the different adverse events profiles of DMTs, and the patient’s preference, with the idealist goal to reach a 100% of therapeutic adherence (9, 10). Some patients do not receive DMTs for years after diagnosis and medication can no longer help as their disability is worsening. Patients with MS now have a longer life expectancy, in part because of earlier treatment. Furthermore, taking into account age-related vascular comorbidities will become increasingly important in patient management (2, 11, 12).

In MS therapeutic reality we have so far limited possibility to predict the DMT efficacy for each patient, because we lack DMT-specific biomarker(s) of efficacy or failure, and the challenge is represented by when to start, when to change, when to stop any of licensed DMT (13-16).

The most used therapeutic approaches for MS are escalation and induction (17-19).

Escalation therapy is an early start with the so defined first-line DMTs (beta interferon, glatiramer acetate, teriflunomide (TRF), dimethyl fumarate (DMF) and fingolimod (FYG) (in many countries out from Europe) and if such DMTs are ineffective or partially effective, switching to more aggressive treatments, defined as second line DMTs (mitoxantrone, natalizumab (NTZ), alemtuzumab (ALEM), ocrelizumab (OCR), cladribine (CLAD), and

FYG (in Europe) (6). The induction strategy immediately pursues higher efficacy, since drugs with a higher risk profile are used from the outset (20).

In this way, the immunosuppressive DMTs, are used to hit the immune system early and hard with the idealistic goal of striking the autoimmune activities before the neurological damage becomes irreversible.

In this review, we will focus on the current role of immunosuppression in the management of the relapsing remitting form of MS (RRMS), moving from the rationale of its use and looking at the futuristic possibility to design an idealistic scenario of a personalized approach for each single patient.

2. Methodology section

The search strategy used follows PRISMA search guidelines (21). The flow diagram of study selection is presented in Figure 1.

Research is updated at the index date of May 30th, 2019 and we investigated the following databases: PubMed, Web of Science and Scopus.

A comprehensive literature search was carried out by two of the authors (AZ and ED) to find articles that investigated personalized therapy and immunosuppression in MS. The reviewers were blinded to each other's and were under the supervision of another reviewer (FP).

The key search terms were the following: immunosuppression, Multiple Sclerosis, personalized, treatment. AND' 'OR' Boolean operators joined the search terms within groups.

Research was restricted to English language and people with MS. All the studies were classified according to American Academy of Neurology (22). A total of 2,017 non-

duplicate citations articles were screened from database searches, with 126 finally included (see Figure 1).

3. Principles and consideration for RRMS therapeutic strategies

The majority of MS pathogenesis studies have been conducted on T cells so far, specifically focused on CD4+ T cells, but more recently, much effort have been exerted to study the role of other immunological cells as CD8+ T cells, and B cells; as well as NK cells and neutrophils (23).

Overall, it seems that any dysregulation in one or more of these cells' subset may occur singularly or in combination, and that could explain some possible variation in therapeutic efficacy.

MS consists of an overlap of inflammation and neurodegeneration, with the inflammatory component more active early (at least in white matter plaques), and the degenerative persistent from the outset, but more prominent as aging is superimposed on the MS-affected nervous system and inflammation changes in the central nervous system (24). Based on this evidence, the rationale of DMTs use is to influence the initial inflammatory phases, so to delay as far as possible the subsequent chronic phase.

Inflammation is the unique target of our available immune therapy and any realistic therapeutic strategy should be based on impacting the neuroinflammation with a range of possibilities, which move from a non-selective immunosuppression to highly specific immune modulation (25-29).

Change of treatment paradigms in MS is linked to growing therapeutic armamentarium with a range of highly active immunotherapeutic drugs, commencing with the first monoclonal antibody NTZ in 2006. Although there is still no cure available and no clearly

accepted disease pathogenesis, the chronic and long-lasting nature of MS, the idea to understand and label the disease at the onset could be particularly challenging for the long-term prognosis of patients with relapsing forms of the disease. The improved treatment options and, in particular, the availability of highly effective therapies, great effort has been posed to define those clinical and paraclinical features which should allow to define a patient as highly active or not, and in recognizing early signs of suboptimal response to DMTs (Figures 2-3) (30).

Therefore, the definition of what is a treatment success has been redefined. Different combined scores to measure disease activity are currently employed; one of them is 'no evidence of disease activity' composed of absence of relapses, MRI activity and disease progression. Long-term data from clinical trials underscore the importance of early immune therapy on disease progression and long-term outcomes. Therefore, MS should be treated as early as possible, and treatment efficacy should be monitored continuously.

However, the emergence of DMTs with high efficacy is accompanied by an extensive management of increasing array of adverse events; so, a deep knowledge of each DMT immunological target and safety profile is essential.

The degree of selectivity for MS treatment is limited and classically the existing therapeutic approaches modulate the immunological response with general or selective immunosuppressive strategies, specific regional strategies or altering immune cells regulation (31, 32).

4. Immunosuppressant therapies in RRMS

The ideal MS therapy would selectively restore failed immune tolerance without impeding other parts of the immune system. Most immune therapies for MS are associated with immunosuppression, which is typically defined as an inhibition of the adaptive immune

system. This definition refers to both short-term/intermittent (pulsed, induction) and long-term persistent immunosuppression (chronic, maintenance)(33). A practical way for a drug to be considered an immunosuppressant is whether the observed effects include one or more of: 1) direct cytotoxic activity or suppression of haematopoiesis 2) lymphocytes' trafficking and the immune surveillance.

Immunosurveillance is the constant process whereby immune cells are trafficking around the body and looking for target antigens, for foreign invaders such as bacteria, viruses, fungi, and other foreign substances to attack. The clinical experience with DMTs use in MS reality gave the possibility of a broader definition of immunosuppression, and it has been proposed that any DMTs which is able to increase the susceptibility to opportunistic infections or neoplasms, could be considered immunosuppressant (34-36).

4.1 Old immunosuppressant

In this category we could include DMTs such as azathioprine, cyclophosphamide, mitoxantrone (which is FDA approved for MS), and methotrexate, which has been used for some time to potentially slow the progression of MS in a low-dose weekly form, but as an off-label use.

Azathioprine is an immunosuppressive DMT which antagonizes purine metabolism and may inhibit synthesis of DNA, RNA, and proteins. It may also interfere with cellular metabolism and inhibit mitosis, causing chain termination and cytotoxicity. It has been used for RRMS until the first half of the 1990s principally as an adjunctive form of therapy, and in some instances, as a first choice treatment for those unwilling to use interferon beta or glatiramer acetate, despite somewhat conflicting data from several studies regarding its ability to reduce relapses and the uncertainty of its effect on disability progression (37-40).

Cyclophosphamide is an alkylating agent related to nitrogen mustard that binds to DNA and disrupts cell replication. It has been studied as a treatment for MS for the past 40 years and many reports suggest that it is efficacious in cases of worsening RRMS. The most widely used regimen is monthly pulsed therapy with 800 mg/m2 administered monthly for 1 year, followed by bimonthly treatments in those who are responders, although numerous other regimens have been proposed (30). The safety profile for cyclophosphamide is well-established. Aside from the anticipated side effects of nausea, vomiting, alopecia, transient immunosuppression, and amenorrhea that are commonly observed in this therapeutic class, the most common general causes for concern are haemorrhagic cystitis, gonadal toxicity (in both men and women), bladder cancer. The risk of bladder carcinoma appears to be associated with cumulative exposures of >100 g and possibly related to duration of exposure (2.7 years) (41).

Mitoxantrone is synthetic anthracenedione – a cytotoxic agent that inhibits DNA repair via inhibition of topoisomerase II leading to a suppressed proliferation of T cells, B cells, and macrophages, decreased pro-inflammatory cytokine secretion, enhanced suppressor T cell function, and suppressed macrophage-mediated myelin degradation (42). It is approved for use in several European countries in patients with worsening RRMS, secondary progressive MS and relapsing-progressive MS in doses of 12 mg/m2 of body surface area every 3 months (43). Concerns have been raised about secondary acute leukemias, which have been reported to occur in approximately 0.8% of mitoxantrone-treated patients in randomized, controlled trials (44), however, this may be an underestimate based on the potentially long latency period of secondary leukemia and the relatively short duration of the trials (44).

Methotrexate is an anti-neoplastic anthracenedione derivative that is related to the class of anthracyclines. Oral Methotrexate was used in treatment of RR and progressive MS

without significant side effects (45). In some studies, Methotrexate was prescribed as second line treatment of MS or as combination therapy, but the overall effects of drug in striking MS activity is questionable (45, 46).

4.2 New immunosuppressant DMTs

Alemtuzumab *(ALEM)* is a humanized monoclonal antibody approved in more than 50 countries in Europe; ALEM is indicated for the treatment of adult patients RRMS with active disease (clinical or neuroradiological); in the USA, it is reserved for the treatment of RRMS patients with inadequate response to two or more DMTs (47-49). In clinical trials, ALEM demonstrated efficacy in patients with high disease activity both naïve and no-responders to previous DMTs (50).

It is administered intravenously with a typical scheme: 12 mg per day for five days the first year, with a re-treatment one year later (the same dose) for three days, and as-needed retreatment (3 consecutive days at least 12 months after the last course). A monthly monitoring is required up to 48 months from the last infusion for an adequate safety management (51).

It is a humanized monoclonal antibody against CD52 epitope on the surface of $CD4^+$ and $CD8^+$ T lymphocytes, B-cells, and monocytes. ALEM rapidly and effectively eliminates circulating CD52 cells via antibody and complement-mediated depletion. After administration, circulating lymphocytes are depleted. The kinetic of reconstitution of immune system differ according to cell lineages: monocytes and B-cells are the first to repopulate in the peripheral blood, approximately 3–6 months after treatment. Whilst, T-cell and particularly CD4+ cells reconstitute more slowly, had normal levels after 2–3 years (47).

Efficacy and safety have been studied in phase II and III clinical trials in treatment-naïve patients:

CAMMS223, in patients with high disease activity compared to subcutaneous Interferon beta 1 a,-CARE-MS I, II and in extension (NCT00930553) and long-term follow-up studies (NCT02255656) that include patients from the phase II, III, and IV trials.

Patients treated with ALEM in the two-year studies, 77% (n = 290/376) of the CARE-MS I study and 69% (n = 300/435) of the CARE-MS II study completed follow-up long term up to the eight year. After receiving the first two cycles of ALEM, at the time of the study and 12 months later, 56% (n = 197) CARE-MS I and 44% (n = 172) CARE-MS II of patients treated with ALEM entered the extension, they did not receive further treatments up to the eighth year of follow up (52). The annualized relapse rate observed in patients treated with ALEM in CARE-MS I (0.18) and CARE-MS II (0.26) for 2 years (both $p < 0.0001$ compared to treatment with sc Interferon beta 1a), remained low during the extension (0.14 and 0.18 at the eighth year, respectively). At the eighth grade, 71% (n = 252) and 64% (n = 260) of patients treated with ALEM in CARE-MS I and CARE-MS II, respectively, did not show a worsening of disability; 41% (n = 84) and 47% (n = 135), respectively, had instead a confirmed improvement in disability.

In the eighth year, patients who had received ALEM in CARE-MS I, had a reduction in brain volume loss was observed. In the years from the third to the eighth, the annual loss of brain volume was -0.22% or less, and -0.19% or less, respectively, lower than that observed in patients treated with ALEM (CARE- MS I: -0.59% in the first year, -0.25% in second year, CARE-MS II: -0.48% in the first year, -0.22% in the second year). From the second to the eighth year, most patients had no evidence of disease activity on MRI 4 (66-77% in CARE-MS I and 66-76% in CARE-MS II).

The global safety profile is good, nevertheless, several adverse events of interest have been reported. The most frequently reported were infusion-associated reactions, experienced by >90% of patients (53). The incidence of infections was greatest during the first month following infusion in all three trials; the most common autoimmune event was thyroid disease. Immune thrombocytopenic purpura incidence across all clinical trials was 2% in patients receiving ALEM 12 or 24 mg (1.6% in patients receiving ALEM 12 mg) (54). Then, were introduced a Risk Management Program and a Risk Evaluation and Mitigation Strategy (55) to ensure early detection of potential adverse events.

Progressive Multifocal Leukoencephalopathy (PML) has been reported in one patient treated with TRF (56) and in one treated with ALEM (47) but in both cases, patients have developed PML after switching from NTZ to the new drug, and PML was finally attributed to NTZ.

Recently was described the first case of ALEM related PML occurring in a MS patient, with detailed analysis of immune characterization of the patient (57).

On April 2019, EMA Pharmacovigilance Risk Assessment Committee has advised that use of ALEM be restricted as a temporary measure; it should only be started in adults with relapsing-remitting multiple sclerosis that is highly active despite treatment with at least two DMTs or where other DMTs cannot be used (58). An investigation is underway following new reports of immune mediated conditions and problems with heart and blood vessels with this medicine, including fatal cases(58). Since the initiation of the ALEM clinical development programme, 39 stroke cases had been reported as of July 2018. Of these, nine cases occurred within 48 h of the infusion (58, 59). Ten heart attacks and six arterial dissections were also reflected in the EMA's safety Committee recommendation (58-60). EMA will consider any additional measures necessary to protect patients and

whether there should be changes in the authorized use (58). Patients who develop signs of pathological immune activation should be evaluated immediately, and a diagnosis of hemophagocytic lymphohistiocytosis considered (58, 59).

Cladribine (CLAD) is a deoxyadenosine analogue prodrug that preferentially depletes lymphocytes, key cells underlying MS pathogenesis. CLAD tablets represent the first short-course oral DMT for use in MS. The tablets are administered in two short courses one year apart; It is indicated for the treatment of adults with highly active relapsing MS on the basis of data from pivotal clinical trials (61-64).

In the CLARITY trial, tablets dose of 3.5 mg/kg versus placebo over 96 weeks in adults RRMS demonstrated to reduce clinical relapse, disability progression and MRI-assessed disease activity and also improved quality of life (HR-QOL)(65). Moreover, in the 96-week extension no additional clinical benefit was gained from continuing versus discontinuing CLAD tablets after the first two annual courses of therapy. The post hoc analyses of CLARITY revealed greater benefits in patients with high disease activity RRMS (63, 66, 67).

In the trial ORACLE (ORAlCLadribine in Early MS, a 96-week, randomized, double-blind, placebo-controlled, international trial) were involved more than 616 clinically isolated syndrome patients. CLAD, compared with placebo, significantly delayed the time to conversion to clinically definite MS; the authors reported a 62% risk reduction for 5.25 mg/kg dose (HR 0.38; 95% CI 0.25–0.58; $p < 0.0001$), and a 67% risk reduction for 3.5 mg/kg dose (HR 0.33; 95% CI 0.21–0.51; $p < 0.0001$)(64).

CLAD has shown a good tolerability profile; despite the lymphopenia, overall, the incidence of infections did not differ among treatment groups. However, the risk of cancer (0.34%) was higher than in the placebo groups(66).

Then, to define the long term safety profile, all patients previously enrolled in any clinical trial with CLAD are invited to join the PREMIERE (Prospective Observational Long-term Safety Registry of Multiple Sclerosis Patients Who Have Participated in CLAD Clinical Studies) study(66), with over 10,000 patient years of exposure, in total, with follow-up in some patients exceeding eight years at completion (clinicatrial.gov: NCT01013350)(62).

Data revealed that during a study period of 194 weeks (mean), lymphopenia was reported more frequently in CLAD treated patients than in placebo group. Consequently herpes zoster was reported more frequently in patients experiencing Grade 3 or 4 lymphopenia; no clustering of types of malignancy, and no malignancies commonly associated with immunosuppression were observed during the follow-up period (62)

In July 2016, the EMA accepted for review the marketing authorization application for CLAD tablets as a treatment for RRMS and its positive decision was stated in August 2017. Currently, the trial CLARIFY, a Phase IV study of CLAD Tablets and quality of life is recruiting patients with highly active disease activity (68). An open-label, single-arm, exploratory Phase IV study in centres in Europe and Australia will assess Multiple Sclerosis Quality of Life-54 [MSQoL-54]) and other patient-reported outcomes (Fatigue Severity Scale; Hospital Anxiety and Depression Scale; Treatment Satisfaction Questionnaire for Medication v1.4) will be assessed at baseline, and at 6, 12, 24 months in RRMS patients receiving CT 3.5□mg/kg (68). The study aims to recruit 356 adults with RRMS by 2019. Final data are anticipated in 2022 (68).

Regarding its safety profile, CLAD should not be applied in patients suffering from severe active or chronic infections (tuberculosis, HIV, VZV, HBV and HCV), severe liver or kidney damage or active cancer (33). Blood lymphocyte counts need to be assessed prior to each individual treatment cycle and after treatment onset and if lymphopenia is below 500/µl an active follow up is needed until recovery (33, 69). Patients should be also monitored for infections, especially herpes virus infections, with oral prophylaxis for herpes virus if lymphopenia is below 200/µl (33, 69).

CLAD is contraindicated during pregnancy and contraception must last-up 6 months after cladribine intake. CLAD has also a potential gametotoxic effects, so male patients also need to apply effective contraception until 6 months after CLAD intake.

4.3 Autologous hematopoietic stem cell transplantation

Patients experiencing rapidly worsening MS despite treatments have been candidate to intense immunosuppression followed by autologous hematopoietic stem cell transplantation (aHSCT). The main goal of aHSCT is the total reset of the hemato-lymphopoietic system followed by the infusion of autologous hematopoietic stem cells (70, 71). AHSCT procedure can be divided in five steps: (1) $CD34^+$ hematopoietic stem cells (HSCs) are mobilized, (2) HSC are collected and preserved, (3) immunoablative conditioning, (4) HSC are infused, and (5) post-transplant care-phase.

Approximately 800 patients with MS have been treated with aHSCT worldwide, always in clinical trial settings. In a first phase, studies of aHSCT were reserved to patients with progressive MS but over time, its use has been extended to a broader range of patients. Consensus expert opinion led to the publication of guidelines in 2012 (72), defining ideal patients for the procedure: a)malignant MS, including Marburg-variant MS 2) highly active relapsing MS with frequent relapses and developing focal inflammatory lesions on MRI despite treatment with one or more lines of conventional treatment; or 3) progressive MS

with ongoing and sustained increase in their disability load only if there are concurrent relapses or focal inflammatory lesions on MRI (72-74).Currently, the use of aHSCT in MS reality could be extended to patients with RRMS young (<45 years old), able to ambulate independently, with disease duration <10 years, and at least two clinical relapses in the last year despite the use of DMTs with MRI evidence of concurrent disease activity. Different transplant procedures are in use, but comparative studies are lacking(75, 76). The high dose immunoablation and autologous haematopoietic stem cell transplantation versus mitoxantrone therapy in severe multiple sclerosis (ASTIMS) trial was a multicenter, phase II, randomized trial including 21 patients with progressive or RRMS, compared the impact of aHSCT vs methotrexate on disease activity measured by MRI(75, 77). aHSCT reduced by 79% the number of new T2 lesions as compared to methotrexate (rate ratio 0.21, p = 0.00016) and T1 Gadolineum + lesions (rate ratio = 0.19, 95% CI 0.09–0.41, p < 0.0001) as well as the annualized relapse rate. No treatment group difference was detected in the progression of disability. Early adverse events were considered as expected and occurred at least in 80% of treated cases. Severe adverse events occurred in the aHSCT arm only and resolved without sequelae (77). The most important element is to reduce regimen-related morbidity remains another concern as virtually all patients develop grade 3 or 4 non hematologic toxicities predominantly in the first 30 days after administration of the conditioning regimen(71, 72, 78). Counselling that includes a thorough explanation of the short- and long-term toxicities and their impact is an important component of care. Reducing the intensity of the conditioning regimen results in lower mortality with several large cohort studies reporting no deaths attributable to aHSCT (71, 72, 78). The most reported toxicities are alopecia, neutropenic fevers, sepsis, urinary tract infections, mucositis, and other gastrointestinal toxicities (79). Rarely can occur thyroid disease or immune thrombocytopenic purpura (79). Infertility is common in both males and females, and patients should be offered the opportunity for gamete preservation prior to

starting chemotherapy treatment. Ovarian failure leading to premature menopause is common (77, 79).

5. Immune-targets era and re-definition of immunosuppressive DMTs in the immune-targets era

As descripted before, DMTs increasing the susceptibility to opportunistic infections or neoplasms (basing on their potential for adverse events traditionally associated with immunosuppression), could be considered broadly immunosuppressant.

Continued immunosuppression then inhibits effective viral clearance could culminate in opportunist infection, as PML, associated with reactivation of JC virus (JCV) in patients treated with NTZ, a monoclonal antibody binds to the apha4-subunit of integrins expressed on the surface of all leukocytes except neutrophils (32, 80-82). NTZ efficacy was demonstrated in primary and secondary endpoints in both AFFIRM and SENTINEL trials (83, 84). Here NTZ was associated with an 83 percent reduction in the number of new or enlarging hyperintense lesions on T2-weighted MRI. However, there are no randomized trials comparing NTZ monotherapy directly with other DMTs. Nevertheless, indirect comparisons suggest (but do not prove) that NTZ is as or more effective than other DMTs (85-87).

The most important adverse event in the treatment with NTZ is the occurrence of PML. Whereas the event is comparatively rare it is fatal in up to 20% of cases and results in permanent neurological residua (82). There is currently no established treatment available other than drug withdrawal, frequently combined with plasma exchange. In keeping with the mechanism of action, the most accepted theory is that $CD4^+$ T-cell lymphocytopenia coupled with increases in IL-10-producing regulatory subsets specifically within the central nervous system may concur to JCV reactivation. It is also unclear whether having latent

JCV within the brain prior to immunosuppression confers greater risk than having it in the periphery, as well as how the mutant form of the virus develops (88). New biomarkers of reactivation may help provide for better monitoring strategies to reduce the future incidence of PML. Risk factors for NTZ associated PML include prior immunosuppression, prolonged treatment duration with NTZ (particularly >24 months), and presence of anti-JCV antibodies (which are >98% sensitive in predicting development of PML but very nonspecific) (81, 88). Although the overall incidence is higher in NTZ, also FYG and DMF demonstrated a low risk (89). PML has been reported in one patient treated with TRF, in one treated with ALEM (see before) and in one after the first infusion of OCR (anti-CD20 DMT), but in all these cases, patients have developed PML after switching from NTZ to the new drug, and PML was finally attributed to NTZ (56, 57, 88, 89).

ALEM and anti-CD20 monoclonal antibodies have been associated with PML in non-MS patients, mostly those with hematologic malignancies (90).

Lymphocytopenia associated with these drugs has been inconsistently reported as a risk factor for PML, and monitoring the absolute lymphocyte count has emerged as standard (87, 88, 90).

Lymphocytopenia usually occurs with other DMTs, commonly DMF, and FTY, two oral drugs approved for the treatment of RRMS.

According to Common Terminology Criteria for AEs, lymphocyte counts higher than 0.9×10^9/L were considered non-lymphocytopenic (grade 0), $\leq0.9 \times 10^9$/L were scored as grade 1, $\leq0.8 \times 10^9$/L as grade 2, $\leq0.5 \times 10^9$/L were scored as grade 3, and ≤0.2 were scored as grade 4.

DMF is the methyl ester of fumaric acid is associated with significant lymphopenia (grade 3; absolute lymphocyte count <500) in approximately 5% of patients in the phase III trials,

particularly among older patients and for those taking the drug for more than a year(90-94). Several cases of PML have occurred in patients taking DMF and related fumarate compounds (90-94) all of whom were lymphocytopenic during treatment (95, 96). Lymphocytopenia is a well-known adverse event that affects continuous drug administration because the absolute lymphocyte count is confirmed to be <200/µl, treatment should be interrupted until recovery (97).

FTY, is sphingosine-1-phosphate receptor (S1PR) modulator, reduces the recirculation of auto aggressive lymphocytes (98, 99). After ligation, the sphingosine1P1 receptor is internalized and degraded,which leads to lymphocyte homing in peripheral lymphatic tissues and prevents the invasion of auto aggressive T-cells into the central nervous system without suppression of systemic immune(98, 99). The lymphocytopenia with FTY reverses rapidly and it is closely related to its mechanism of action. (100) Recently, a multicolor flow cytometry and multiplex assays were used to identify up to 50 lymphocyte subpopulations and to examine the expression of multiple cytokines in selected patients treated with FTY or DMF. It was revealed that different treatments can target different lymphocyte compartments and suggests that lymphopenia can induce compensatory mechanisms to maintain immune homeostasis (100).

TRF is the active metabolite of the parent drug, leflunomide, which is converted almost entirely into TRF following oral ingestion and thus TRF has become the focus of development for use in patients with MS, and it is approved as first line oral therapy. TRF inhibits the dihydroorotate dehydrogenase and: inhibits protein tyrosine kinase, cyclooxygenase-2 and downregulates inducible nitric oxide synthase. Although induced lymphopenia is small, it only partially accounts for global effects of TRF (101). Moreover, the effects of TRF on lymphocyte migration, cytokine production, and surface molecule expression persist despite exogenous pyrimidine administration (102, 103). Based on in

vitro data, it has been suggested that the inhibition of protein tyrosine kinase activity might be another mechanism which mediates the immune effects of TRF, although doubts were raised about the very high concentrations required in vivo in mice for this activity to take place (102, 103). However, taking into account differences between human and rodent cell lines, the immune effects of TRF via tyrosine kinase inhibition might be clinically relevant at therapeutic concentrations in humans (102-104).

Among emerging DMTs, the anti-CD20 monoclonal antibody OCR has been approved by FDA and by EMA for the treatment of RRMS and primary progressive MS (105) (106). The infusion-related reactions, upper respiratory tract infections, and oral herpes simplex infections were more frequent with OCR and neoplasms occurred in 2.3% of the patients who received OCR *vs* 0.8% of patients who received placebo. Overall, with the exception of an increased risk of tumors, there was no clinically significant between-group difference in the rates of AEs and serious infections (4, 105-108).

6. Understanding the genetic influence in drug response

Over the last decade, considerable effort has been made to identify pharmacogenetic markers in the field of MS. To date, efforts have been focused on the identification of markers that determine drug response, and there are no published data relating to pharmacogenetic markers to predict adverse drug reactions. A biomarker must be reliable, costs-effective and easy to translate in clinical practice; however, multiple validations steps are needed to commute theory in real-world application. Progress has been made in determining the effects of particular single nucleotide polymorphism, (SNPs) and genes in MS pathogenesis and DMTs' response. All studies had outlined a number of candidate genes and converge on the need of identification of pharmacogenetic markers to accurate phenotyping of the patients and of its adverse events 'risk profile (109).

This is useful for the choice of a therapy efficacy must be associated with tolerability and low risk of adverse events. It is well established that some of the variability in drug response, and the risk of developing a serious adverse event, is due to genetic variations (110), especially some SNPs at the HLA locus (110); in detail MHC class II HLA-DRB1 gene, HLA-DRB1*15:01, which increases risk about threefold whom over 200 SNPs has been described. (111, 112).

Furthermore, treatment efficacy with NTZ was found related with the polymorphisms in *NQO1* and *GSTP1* gene. In detail, patients who carried the wild-type genotype or only one non-wild polymorphism for either gene have possibly a better clinical outcome after receiving the NTZ therapy (113).

This activity of proteins regulating the pharmacodynamic and pharmacokinetic properties of drugs are key contributors to the variability in response to drugs between patients and in the same patients in the course of life. The major enzymes involved in DMTs metabolism are cytochrome P450 (CYP) and polymorphisms in these enzymes can result in either ultrafast or poor metabolism of therapeutic drugs, with increasing risk of lack of efficacy and toxicity, respectively.

This has been applied for the Siponimod (BAF312), a selective modulator of the sphingosine-1-phosphate (S1P) receptor subtypes 1 and 5 (S1P1,5) approved from FDA for the treatment of RRMS in adults, including secondary progressive MS in the active phase, and clinically isolated syndrome. Siponimod is primarily (approximately 80%) metabolized by the polymorphic cytochrome P450 (CYP) 2C9 enzyme for which three functionally distinct phenotypes have been reported: 1) extensive metabolizer (EM) (CYP2C9*1/*1 genotype) – 2) intermediate metabolizer (IM) (CYP2C9*1/*2; *1/*3; *2/*2 genotype) and 3) poor metabolizer (PM) (CYP2C9*2/*3 or *3/*3 genotype)(114, 115). FDA recommended different maintenance doses according to genotypes, in detail: 2 mg daily

in patients with CYP2C9*1/*1, *1/*2 and *2/*2 genotypes and 1 mg daily in patients with *1/*3 and *2/*3 genotypes, whilst has placed the contraindication for patients with the CYP2C9*3/*3 genotype. These results are based on the estimated net effect on Siponimod exposure in the presence of CYP2C9/CYP3A4 inhibitors or inducers after multiple administrations (116).

In the clinical practice, the genotype will be tested during the screening of patients, with a saliva test charged to manufacturer (115).

Therefore, the choice of DMTs would consider also the identification of genetic difference in drug response and long term follow up, studying disease course are needed for their validation, and it is auspicial in the foreseeable future.

7. Conclusions

Several immunosuppressive DMTs are now available for RRMS patients, with an overall good risk–benefit profiles which could impact significantly quality of life for patients with MS which requires lifelong treatment. Each DMT has a specific immune- targets and immune reconstitution timing. The sequencing of DMTs must consider the disease history and trajectories of each single patients, to optimize treatment response and to reduce safety concerns. Still, methodical approaches can yield rational estimates of risks and guide preventive management strategies to recognize suboptimal response or negative prognostic factors when a new DMT is chosen (Figure 4).

8. Expert opinion

The possibility to design a patient-oriented therapy in MS received a boost in the last years, but such possibility was counteracted by new and severe safety alerts.

Several studies converge on the hypothesis that start early and with high efficacy DMTs is associated with better long-term outcomes. The key-point is represented by the accurate selection of patients defining those with high risk of disability progression for the presence of negative prognostic factors at onset.

There are still open questions about the long-term outcomes of initial high-efficacy DMTs both in terms of efficacy and safety, and about the identification of markers to predict long-term treatment responses to high-efficacy DMTs.

It is evident that in the modern era, DMTs have radically changed the long-term impact on time to secondary progressive MS and hard disability outcomes, time to needing a walking stick, and death. It is also been demonstrated that high efficacy DMTs are superior to interferon beta and glatiramer acetate (32, 117, 118).

Based on these observations, starting therapy with the most effective DMTs early is likely to improve disability outcomes further (119). Nowadays, the question is whether we are undertreating the disease. Indeed, an important challenge is the definition of benign MS with great lack of consensus. Expanded Disability Status Scale (EDSS) is biased towards ambulation and cognitive function is relatively ignored. Portaccio et al. performed systematic studies of cognition and neuropsychological function in benign cases (defined as EDSS 3.0 or better 15 years or more from onset) (120). Here, cognitive impairment, in apparently benign form of the disease was associated with worst long-term course. (121). Furthermore, also patients with normal cognitive function may have compromised brain functioning with early structural damage and the progressive accumulation during disease leads to an inevitable decrease in all networks efficiency (intended as recruitment of brain

areas and/or altered connectivity between regions) (122, 123). The impact of highly effective DMTs may decrease as the disease unfolds in line with the natural history of MS, where we can see that the impact of relapses on disability progression is higher in the earlier stages of the disease (124).

The ideal time to consider risk mitigation for MS immunotherapies starts right from the moment of diagnosis, in the very early stages of choosing therapies, considering also planned fertility weighting maternal and fetal risks.

We are attempting to design a specific safety profile for every single patient, basing on strict monitoring programs, which are usually based on the classic haematological parameters.

We are trying to insert in the clinical practice the study of haematological subpopulations, as for B and T lymphocytes, to better define the patients at more risk of infections or haematological complications. Moreover, we should keep in mind that the suppression on immune system may last for an indefinite time in every single patient and no markers of prediction exist for such topic. For instance, we have witnessed to different profiles of the reconstitution of immune system after CLAD and ALEM use. After CLAD, B cells slowly repopulated, remaining significantly below their baseline level until the second treatment cycle reduced this subset yet again. By contrast, B-cell numbers after ALEM repopulated back to baseline within less than 6 months and then hyper-repopulated above baseline by 9 and 12 months (125). And the registrative phase III trials only reported that B cells reach normal levels 6 months after drug administration. Is that related to the recent reports of new and previously unknown cases of autoimmune disease (thyroiditis, autoimmune purpura, diabetes etc...) reported after ALEM use? We need clarification.

The ideal therapeutic strategy should promote immune reconstitution very early without impairing natural defense mechanisms or endogenous control of incipient autoimmunity to maximize benefits and reduce risks.

Regarding HSCT, emerging studies are showing impressive results on the ability to control an aggressive MS course, but so far, patients who are candidate to such aggressive treatment are selected basing exclusively on the disease activity and not on their immune system profile. Why in some MS patients knocking out the immune system might work better than drugs are still unknown.

Furthermore, patients with MS over 55 years are increasing and the risks of high-efficacy DMTs must focus on age-related efficacy and risks, including opportunistic infections, malignancies, and autoimmune reactions. It is hypothesized that age-related and therapy-induced alterations to the immune system may have (super)additive effects, resulting in an acceleration of physiological immunosenescence and inflamm-aging (126). The regenerative potential of the brain is limited and becomes less effective with age (116).

All these suggest that swift action to prevent or slow damage to the brain is crucial. Clinicians must act before the disease causes.

We pose great expectations on the pharmacogenetic studies which could help in discovering the individual genetic background that underlies the heterogeneity of treatment response, and so help in finding biomarkers for identification of patients who will benefit the maximum from the therapy administered, and could help in foreseeing adverse event, in particular the hematological complications.

MS could represent an ideal disease for a personalized treatment approach for the wide range of clinical presentation and therapeutic responses, and we hope that in the next

future we could build different models of treatments to optimize the benefits of DMT for individuals, while minimizing the risk of adverse events.

We have moved away from one size-fits-all therapy to treatment algorithms with greater emphasis on individual attributes, but only when we answer these remaining questions will we fully enter the era of personalized medicine in MS.

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****It provides useful information about personalized medicine.**
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****It provides useful information about age and DMTs' use in MS patients.**

Figure legends

Figure 1. PRISMA flow diagram

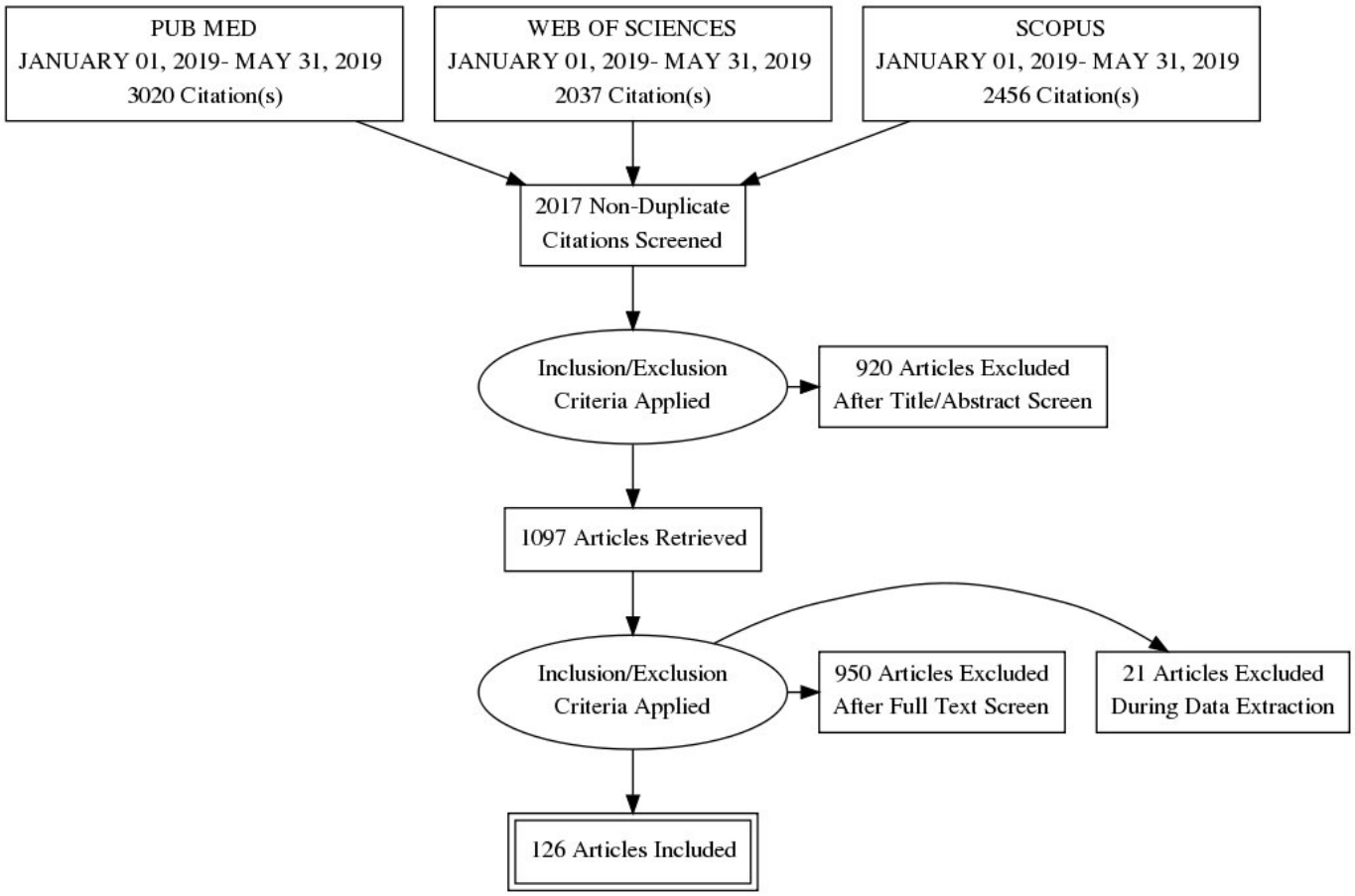


Figure 2. Negative prognostic factors at MS onset

EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; MS, Multiple Sclerosis

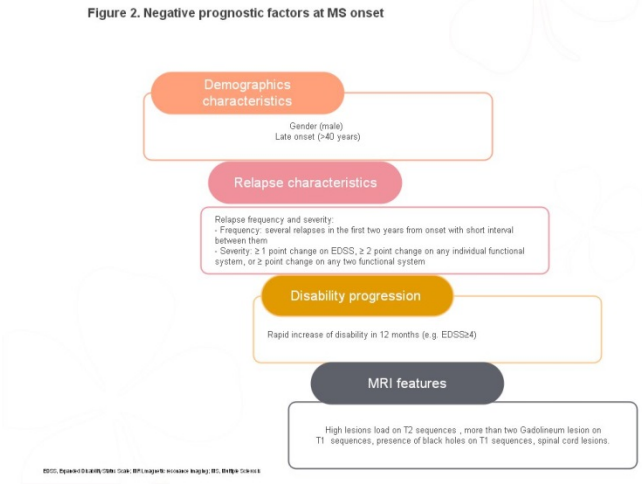


Figure 3. Considerations for failure or loss of efficacy of DMTs in RRMS

CSF, cerebrospinal fluid; DMT, disease modifying treatment; EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; MS, Multiple Sclerosis; RR, relapsing remitting.

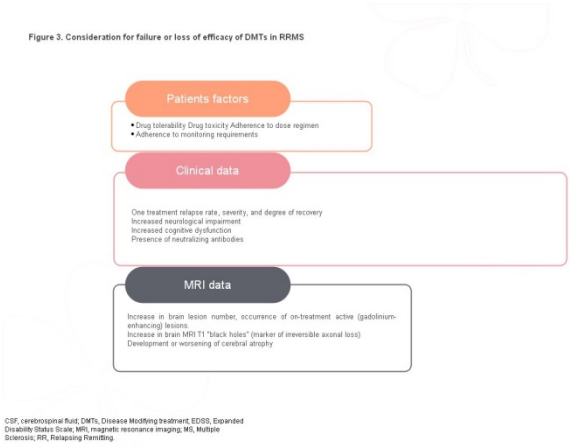
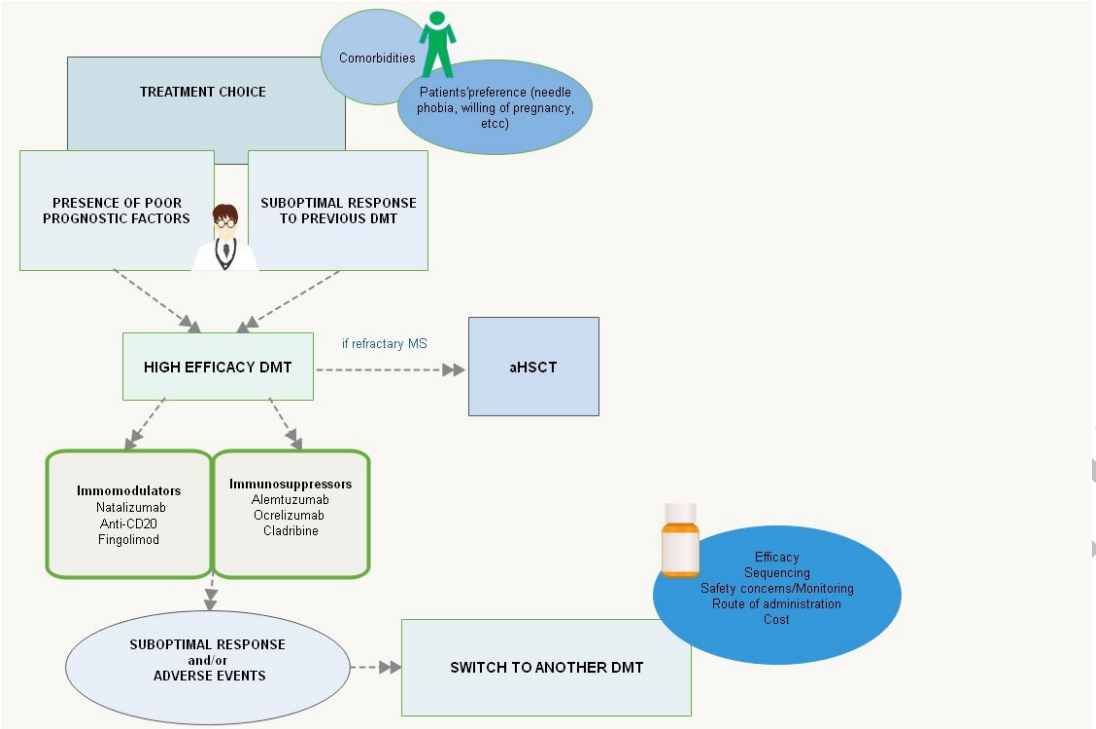


Figure 4. From immunosuppression to personalized therapy in RRMS

aHSCT, autologous hematopoietic stem cell transplantation; DMT, disease modifying treatment; MS, Multiple Sclerosis; RR, relapsing remitting



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