**UNIVERSITA' DEGLI STUDI DI GENOVA**

Dottorato in Neuroscienze XXXV ciclo

# Impact of highly active immunotherapy on acute and chronic neuroinflammation in aggressive multiple sclerosis

**Supervisor: Prof. Matilde Inglese**

**PhD Candidate: Dr. Giacomo Boffa**

*“Mi avete fregato di nuovo”*

***Lettera a una professoressa***

***Scuola di Barbiana***

***Firenze 1976***

# INDEX

# 1. INTRODUCTION

## 1.1 Multiple sclerosis: treatment goals and unmet needs

Multiple sclerosis (MS) is the most prevalent chronic autoimmune-driven neurodegenerative disorder of the central nervous system (CNS), affecting more than 2 million people worldwide. The disease remains a major public health issue as the leading cause of non-traumatic neurological disability among young adults. The ultimate cause of MS is unknown, but several genetic and environmental risk factors have been proposed (Human-Leukocyte-Antigen DRB1*1501 haplotype, geographic latitude, Epstein-Barr-Virus infection, vitamin D deficiency, tobacco exposure and intestinal microbiome composition) (Reich et al., 2018; Thompson et al., 2018b). The key neuropathological hallmarks of MS include demyelination, inflammation, astrocytic gliosis, and neurodegeneration, which is considered the main pathological substrate of clinical progression. Clinically, MS is a very heterogeneous disorder and main symptoms include unilateral optic neuritis, partial myelitis, sensory disturbances, brainstem syndromes and cognitive impairment. Based on the 2017 revised McDonald criteria (Thompson et al., 2018a), a diagnosis of MS can be reached on clinical assessment alone or with the combined use of magnetic resonance imaging (MRI) and cerebrospinal fluid assessment, demonstrating the dissemination in time and space of the inflammatory process. Traditionally, multiple sclerosis has been categorized as relapsing-remitting multiple sclerosis (RRMS), secondary progressive multiple sclerosis (SPMS), and primary progressive multiple sclerosis (PPMS) (Lublin et al., 2020, 2014). However, recent evidence suggests that the clinical course of multiple sclerosis is better considered as a continuum, with boundaries between the different phenotypes being somewhat indistinct. The evolution to a progressive course is thought to reflect a shift from localized acute injury to widespread inflammation and neurodegeneration, along with the concomitant failure of compensatory mechanisms, such as neuroplasticity and remyelination (Kuhlmann et al., 2023).

With one of the most impressive series of new disease modifying therapies (DMTs) approved over the past decades, MS represents a great story of bench-to-bedside success. Today more than a dozen DMTs are available, with different mechanisms of actions and efficacy/safety profiles (McGinley et al., 2021). The introduction of newer, more efficacious, therapies have changed the natural history of the disease, delaying the accumulation of severe neurological disability in most patients (He et al., 2020).

Nevertheless, there are still several unsolved problems in MS treatment.

First, MS remains very challenging to prognosticate. Although several clinics, demographic, immunologic and radiologic risk factors of worse outcomes have been identified, the disease course is highly heterogeneous and it is difficult to early identify patients who will develop an aggressive form of MS (Malpas et al., 2020). Second, long-term complete MS remission remains elusive in real life and the question of whether treatment can fully prevent long-term clinical progression is controversial (Rotstein et al., 2015). No-evidence-of-disease-activity, consisting in the absence of inflammatory MRI activity, clinical relapses, and disability progression (NEDA-3 status), has been proposed as a reasonable, although ambitious, goal in MS patients treated with active DMTs (Parks et al., 2017). However, in real-world studies, most MS patients do not maintain NEDA-3 status for longer than 2 years, despite the use of new high efficacy DMTs (Sormani et al., 2017a). Studies with longer follow-up have shown that more than half of treated RRMS patients accumulate relevant disability over 10 years, mostly independent of 2-years NEDA-3 status (Cree et al., 2019, 2016), questioning the utility of these metrics as outcome measures in clinical trials. The subtle progression which occurs in relapse- and MRI activity-free patients has been described as progression independent of relapse activity (PIRA), and it is emerging as one of the main drivers of disability progression in all phases of the disease (Giovannoni et al., 2022; Kappos et al., 2020). A shift away from simply targeting relapses and focal MRI activity is needed, with the focus of attention redirected to halting the putative processes responsible for smoldering MS.

Finally, to date, no medication has been convincingly demonstrated to promote remyelination or neuronal plasticity (directly or indirectly), thus reversing impaired neurologic functions. Although some DMTs have been associated with transient neurological improvement, the clinical relevance of these findings is little and needs to be further evaluated.

## 1.2 Aggressive multiple sclerosis

Aggressive multiple sclerosis is characterized by severe relapses, accelerated accrual of disability and poor response to treatment (Iacobaeus et al., 2020). There is no universally accepted definition of aggressive MS and several different criteria have been used. According to the different definitions, it is estimated that aggressive multiple sclerosis develops in 4-14 % of patients, meaning about 6.100-14.600 people in Italy. An early attempt to define this group of patients was “malignant multiple sclerosis”, which was defined as “disease with a rapid progressive course, leading to significant disability in multiple neurologic systems or death in a relatively short time after disease onset” (Lublin and Reingold, 1996). To overcome the limits of this definition, which appeared somewhat vague, other authors proposed to classify as “ever malignant multiple sclerosis”, patients who reached an Expanded-Disability-Status-Scale (EDSS) score of 6 within 5 years from disease onset (Gholipour et al., 2011). These patients had significantly more relapses, more motor symptoms, and a higher frequency of a progressive onset. According to Menon et al. (Menon et al., 2017), aggressive MS could be defined when: (i) patients who reached an EDSS of 6 within 5 years from disease onset; or (ii) patients reached an EDSS score of 6 before they are 40 years old; or (iii) patients converted to a secondary progressive MS phenotype within 3 years from disease onset.

These definitions of aggressive MS are however hampered by the need for either a retrospective assessment of the disease course or a prolonged prospective evaluation until fulfilling the proposed criteria and thus hinder the ability to make rapid and efficient treatment decisions.
Rush et al. defined aggressive MS as relapsing MS with one or more of the following features: (a) EDSS score of 4.0 within 5 years of onset; (b) Multiple ($\geqslant$2) relapses with incomplete resolution in the past year; (c) $\geqslant$2 MRI scans showing new or enlarging T2 lesions or Gd+ lesions despite treatment; (d) No response to therapy with one or more DMTs for up to 1 year (Rush et al., 2015). Other definitions of aggressive MS have been proposed when considering treatment options. Edan et al. (Edan et al., 2011), organizing a clinical trial on the inductive effect of mitoxantrone, defined “aggressive MS” as $\geqslant$2 relapses or an EDSS increase $\geqslant$2 points in the 12 preceding months, $\geqslant$1 Gd-enhancing lesion and baseline EDSS between 2.5 and 5.0. Saccardi et al. (Saccardi et al., 2012), aiming at identifying patients eligible for hematopoietic stem cell transplantation, defined “highly active MS” as “failure of at least one and up to three active DMT evidenced by ongoing or increased clinical and MRI activity”. A recent registry-based study identified early and clinically accessible

makers that, if observed in the first year since symptom onset, convey increased risk of the patient meeting criteria for aggressive disease (EDSS of 6 within 10 years from disease onset)(Malpas et al., 2020). Specifically, (i) older age at symptom onset, (ii) greater disability in the first year, and (iii) the presence of pyramidal signs, are associated with aggressive multiple sclerosis.

Some studies tried to identify baseline MRI characteristic predictive of a later aggressive MS course. Tintoré et al. used a Barcelona-based inception cohort and defined aggressive MS as reaching an EDSS $\geqslant$6.0 within 10 years of disease onset (Tintore et al., 2020). Early radiological biomarkers associated with this outcome were the presence of $\geqslant$20 lesions on T2-weighted images or $\geqslant$2 gadolinium enhancing (Gd+) lesions. In another study, the presence of $\geqslant$2 Gd+ lesions and $\geqslant$1 spinal cord lesions at baseline and 1 year and $\geqslant$1 new spinal cord lesions at 3 years of disease evolution were associated with conversion to SPMS at 15 years (Brownlee et al., 2019a). A study in CIS patients showed that a percentage brain volume change (PBVC) decrease below −0.817% in the first year of disease evolution was an independent predictor of a shorter time to a second attack (Pérez-Miralles et al., 2013).

No evidence-based criteria exist to guide the choice on the best treatment approach in these patients. The recently updated/published guidelines from the American Academy of Neurology (Rae-Grant et al., 2018) and the European Academy of Neurology/European Committee on Treatment and Research in MS (Montalban et al., 2018) do not address the management of patients with aggressive MS. For some available DMTs, regulatory approval and insurance coverage mandate that patients must be considered not responsive or intolerant to platform, low efficacy therapies before receiving access to more effective treatments. This strategy is debatable in patients with a more aggressive form of disease, in which the successful therapeutic window of opportunity may be narrower than for those with less aggressive disease. Patients with aggressive MS are underrepresented in typical randomized clinical trials, due to the strict inclusion/exclusion criteria of sponsored clinical trials and the ethical dilemma of blind randomization of a patient with such an aggressive disease. It is therefore necessary to collect data on the best treatment strategy in patients with aggressive MS, in terms of onset of action, intensity of the immunosuppressive effect and the long-term outcomes.

## 1.3 Hematopoietic stem cell transplantation

Intense immunosuppression followed by hematopoietic stem cell transplantation (HSCT) has been increasingly explored as a treatment strategy for aggressive MS. The concept of HSCT in severe autoimmune diseases initially evolved in animal models, with the potential benefits in humans supported by reports of profound clinical response to HSCT performed for other conventional indications. Starting from 1997, the first autologous HSCT (AHSCT) procedures specifically for MS were performed, and more than 1800 MS patients have now been treated worldwide. The rationale of AHSCT in MS is to eliminate self-reacting cell clones and to induce self-tolerance through a profound renewal of the immune system. Numerous biological mechanisms are thought to be responsible for the resetting of the aberrantly overactive immune response in MS, including the normalization of the restricted T-cell repertoire in peripheral blood and in the CSF, the selective reduction of the encephalitogenic effector response, the increase of the immunoregulatory cell subsets and the reduction of the pathogenic memory cells within the bone marrow niche, which are thought to drive chronic inflammation (Cencioni et al., 2022). Haematopoietic stem cells can be obtained either from the affected patient (autologous) or from a closely matched donor (allogeneic). Allogeneic HSCT is rarely used in the treatment of immune-mediated neurological disorders, due to the risk of severe, life-threatening adverse events.

*The procedure*

Briefly, the AHSCT procedure involves several stages: pretransplant optimization, stem cell mobilization and collection, conditioning chemotherapy, stem cell reinfusion, and post-transplant supportive care. An extensive pre-transplant evaluation of fitness for transplantation including echocardiography, pulmonary function testing, spleen ultrasonography, chest X-rays and blood testing including an infection screen, must occur. In addition to this, counseling of patients regarding the risks of transplantation is required, particularly death, serious adverse events, infection, autoimmune disease, and infertility. Indeed, since three quarters of people with MS are women and MS age of onset is usually between 20 and 40 years old, women of childbearing age are overrepresented in the population of potential candidates for AHSCT. The potential for fertility loss and premature menopause associated with AHSCT are therefore significant issues for patients with MS. Although preliminary data suggest higher menstruation resumption rates in autoimmune patients treated with AHSCT compared to the onco-hematological setting and some pregnancies

after AHSCT for MS have been reported (Chatterton et al., 2021; Massarotti et al., 2021; Snarski et al., 2015), the available evidence does not address a proper risk evaluation.

In MS patients, peripheral blood hematopoietic stem cells (PBSC) are usually mobilized with cyclophosphamide (CY) at a variable dosage of 1.5–4 g/$m^2$, associated with uromixetan and hyperhydration, followed by daily granulocyte colony-stimulating factor (G-CSF, 5–12 μg/kg/day). Since urinary bladder dysfunction is common in MS, residual volumes of urine represent a risk factor for infection and for hemorrhagic cystitis due to CY metabolites. PBSC could be theoretically mobilized with G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) alone, but treatment with CY is preferred in patients with autoimmune disorders, because colony stimulating factors can induce inflammatory flares (MS relapses and/or MRI activity), especially in people with highly active disease (Openshaw et al., 2000). Moreover, CY (at dosages between 1.5-4 g/$m^2$) depletes circulating autoreactive lymphocytes, which could otherwise be collected in the graft(Dubinsky et al., 2010). The procedure can usually be carried out as an outpatient regimen, but in disabled patients, hospital admission is preferred. Mobilization is almost always successful in MS patients (Kyrcz-Krzemień et al., 2016), although some concerns have been raised for MS patients previously treated with mitoxantrone. The surface antigen CD34 allows collection of PBC from the peripheral blood using leukapheresis. To ensure a good transplant with adequate recovery of bone marrow functions, a minimum of 4–5×$10^6$ CD34+ PBSC/kg body weight is recommended. Doses higher than 8 × $10^6$/kg are unlikely to improve the rate of engraftment and have a theoretical risk of increased T cell contamination of the graft. The graft can be manipulated to further purify CD34+ cells, but the ex vivo graft selection increases the risk of infections and raises the costs of AHSCT and it is not recommended by the EBMT. After 30–60 days, the patient is treated with a conditioning regimen which has the aim of eliminating the autoreactive clones in the immune system. Different conditioning protocols have been proposed and classified according to the intensity of the haemato-lymphopoietic system ablation.

According to the EBMT (Sharrack et al., 2020), conditioning regimens are divided into the following:

1. High-intensity regimens, as busulfan + CY + rabbit anti-thymocyte globulin or total body irradiation + CY + ATG
2. Intermediate intensity regimens, which can be further classified as:
    1. Lympho-myeloablative such as BEAM+ATG, consisting of BCNU 300 mg/$m^2$, cytosine-arabinoside 200 mg/$m^2$, etoposide 200 mg/$m^2$ and melphalan 140 mg/$m^2$ + ATG 5-7.5 mg/kg

2. Purely lymphoablative such as CY 200 mg/kg + ATG 5-7.5 mg/kg
3. Low-intensity regimens such as CY 100 mg/kg alone (without ATG nor rituximab)

The optimal intensity of the conditioning regimen for AHSCT in MS remains unknown. Intermediate intensity conditioning using CY+ATG (purely lymphoablative) and BEAM+ATG (myeloablative) appear to be the most widely used, with both demonstrating promising efficacy and both suggested as the most evidenced options by the EBMT (Sharrack et al., 2020). High-intensity busulfan- or total body irradiation-based conditioning protocols are associated with an increased occurrence of death without clear benefit to efficacy and are now rarely used. Low-intensity conditioning regimens are used in some countries, even though the term AHSCT in these cases seems inappropriate because the very low dose of chemotherapy of these protocols does not necessarily require stem cells reinfusion.

After the conditioning regimen, the cryopreserved graft is thawed, and cells infused through a central venous catheter. ATG is administered during the 2-4 days following PBCS infusion and its use is complicated by several serious allergic reactions, including fever (premedication with corticosteroids is usually performed). Recovery of cell counts occurs usually in 7–15 days, during which patients need supportive care (usually transfusions and antibiotics). Generally oral prophylaxis should cover fungal infections, herpes virus and pneumocystis infection for a minimum of 6 months post-AHSCT, with many units extending to 12 months. Viral reactivation is important so PCR-based Epstein-Barr-Virus (EBV)/Cytomegalovirus (CMV) monitoring is recommended during the first 100 days.

After AHSCT, patients do not usually receive any DMT and are monitored clinically and with MRI. Unfortunately, no immunological biomarkers of tolerance induction have been found yet which could help clinicians in monitoring patients after AHSCT and prompt maintenance therapy introduction in high-risk individuals. Analyses from the HALT-MS trial (Nash et al., 2017) failed to show differences in lymphocytes repopulation dynamics and T-cell receptor repertoire between patients with complete disease remission and those with disease reactivation (Harris et al., 2020, 2018). In many patients, MS reactivation after AHSCT can be easily controlled with low-efficacy platform DMTs (Boffa et al., 2021). To date, no PML cases are reported after AHSCT. However, PML is a concern when natalizumab is considered as a DMT in case of MS reactivation after AHSCT (Frau et al., 2018).

*Evidence of efficacy*

Most of the available studies on AHSCT in MS are non-randomized, uncontrolled clinical trials, generating low levels of evidence, reporting data from different heterogeneous transplantation regimens and patient populations (Willison et al., 2022). The first studies mainly focused on the feasibility and the safety of the procedure and enrolled patients with advanced progressive MS. 78% of 281 patients with MS who underwent AHSCT between 1996 and 2005 had progressive MS with a median EDSS score before the procedure of 6.5 (Muraro et al., 2017). These first pioneering studies demonstrated that AHSCT was able to completely abolish MRI inflammatory activity (Mancardi et al., 2001) and clinical relapses, but was not able to fully prevent disease progression in long-lasting inactive progressive MS(Burt et al., 2003). In line with these observations, subsequent AHSCT trials have included predominantly patients with treatment resistant RRMS and ongoing CNS inflammation. Burman et al. reported a 5-year disease free survival of 68% in 34 RRMS and 7 SPMS patients treated with BEAM+ATG or CY+ATG AHSCT (Burman et al., 2014). These results were confirmed and extended by Burt et al. who reported, in a cohort of 123 RRMS and 28 SPMS patients, a four-year relapse-free survival of 80% and a progression-free survival of 87% after CY+ATG AHSCT (Burt et al., 2015). In 2016, Atkins et al. reported efficacy and safety outcomes of 24 patients (12 with RRMS and 12 with SPMS) treated with a high-intensity regimen made of busulfan+CY+ATG (Atkins et al., 2016). MS activity free survival at 3 years after transplantation was almost 70% and MS inflammatory activity (new/active brain lesions and/or clinical relapses) was completely abolished up to 13 years in all patients (Atkins et al., 2016). The 2017 HALT-MS trial (Nash et al., 2017), which included RRMS patients treated with a BEAM+ATG based AHSCT, showed a 5-year progression free-survival of 91.3%, with 62% of patients experiencing EDSS improvement at last follow-up. In the last few years, several uncontrolled studies have been published from all over the world, confirming these results in independent cohorts of patients with RRMS and progressive MS(Chen et al., 2012; Kvistad et al., 2019; Moore et al., 2019). A meta-analysis considering all clinical studies on AHSCT in MS until 2021 (Nabizadeh et al., 2022), showed that globally 81% of patients remained relapse-free 5 years after AHSCT, while new MRI lesions appeared in only 8% of patients, with an overall event-free survival of 63%.

To date, only two randomized, controlled trials have been published. The ASTIMS study (Mancardi et al., 2015) was a randomized controlled phase II trial comparing transplant with an intermediate myeloablative conditioning protocol (BEAM+ATG) versus mitoxantrone, and included a total of 21 patients, of whom 9 underwent bone marrow transplant. Most patients had SPMS and, after a mean follow-up of 4 years, 57% of patients experienced worsened disability. Patients who received AHSCT

did have significantly fewer MRI lesions compared to mitoxantrone and a reduced annualized relapse rate, with no difference in disability progression between groups. The 2019 phase III MIST study(Burt et al., 2019a) included 110 RRMS patients and randomized them 1:1 to AHSCT with an intermediate intensity lymphoablative regimen (cyclophosphamide+ATG) or to the best available platform DMT. Progression occurred in 3 of the AHSCT patients and 34 of the DMT control group, with the EDSS in the AHSCT group at 2 years stable or improved in 94.5%. NEDA at 5 years was 78.5% in the AHSCT group compared to 2.97% in the DMT group. It is important to mention here that the control DMT group included a high proportion of patients treated with glatiramer acetate or the IFN-β drugs and few with highly effective therapies such as natalizumab or fingolimod. No patients in the control group were treated with B-cells depleting agents nor alemtuzumab. Three independent retrospective real-world studies investigated the effect of AHSCT (both CY+ATG and BEAM+ATG conditioning) versus that of alemtuzumab(Boffa et al., 2020; Häußler et al., 2021; Zhukovsky et al., 2021). The results of the three studies are largely concordant and found that AHSCT significantly reduced the risk of relapses and MRI activity, allowing NEDA-3 status in a higher proportion of aggressive MS patients compared with alemtuzumab. Interestingly, in all studies, 28-46% of the patients treated with alemtuzumab had an autoimmune adverse event, compared with 12-20% in the AHSCT group.

Since MS-related disability might take many years or decades to develop, very long follow-up periods are required to understand the exact role of any treatment for MS. Some studies have reported the long-term follow-up (often more than a decade) of transplanted MS patients. Fassas et al. report the long-term follow-up of their pioneering study, showing that disease progression-free survival at 15 years is 44% for patients with active MS (Fassas et al., 2011). Similar results were confirmed by Boffa et al. (Boffa et al., 2021), who reported the long-term follow-up of 210 MS patients treated with several intermediate conditioning regimens and observed that 65.5% of patients were free of disability worsening 10 years after transplantation, with a disability worsening–free survival >70% in patients with RRMS. Muraro et al. (Muraro et al., 2017) analyzed data of 281 patients from the EBMT/Center for International Blood and Marrow Transplant Research (CIBMT) registries and showed that 73% of RRMS patients are free of disability progression 5 years after AHSCT. In the Moscow study(Shevchenko et al., 2015), cumulative incidence of disease progression was 16.7 % at 8 years after a reduced intensity BEAM like AHSCT. Estimated event-free survival at median follow-up of 48.9 months was 80% (83.3 % in relapsing-remitting MS). The experience from Prague (Krasulová et al., 2010) shows that 70.8% and 29.2% of patients were free

of progression at 3 and 6 years after AHSCT. Although heterogeneous, these large cohorts are largely concordant, mirroring the results of more recent prospective trials, suggesting that the effect of AHSCT is long-lasting and persists for over a decade in more than half of aggressive MS patients, without the need of maintenance therapy.

Several independent groups have observed sustained EDSS score reduction after AHSCT in patients with RRMS (Boffa et al., 2021; Burt et al., 2019b; Nash et al., 2017). It is important to note here that most transplanted patients experienced MS attacks right before AHSCT, and the reduction in disability could represent the expected gradual recovery from relapses. However, in some studies EDSS scores continued to ameliorate beyond the first year after AHSCT, when recovery from relapses no longer occurs, suggesting a robust effect of AHSCT in improving neurologic status. Although the mechanisms underlying CNS repair are not completely understood, one of the biggest challenges for recovery seems to be the presence of a chronic inflamed microenvironment impairing remyelination and neuronal plasticity, which could be potentially targeted by the CNS-penetrant chemotherapy used during AHSCT.

*Safety*

AHSCT is a procedure complicated by serious expected side effects. These include mainly febrile neutropenia, mucositis, electrolyte disturbances, anemia, neutropenia, low platelet count and viral reactivation. Fever during neutropenia is almost universal during AHSCT. In the absence of neutrophils which are responsible for most clinical signs or symptoms during an infection, fever is frequently the only symptom present. On the other hand, fever is a highly unspecific sign, and it can be caused by drug reactions (mainly ATG), transfusion reactions, mucositis, and engraftment syndrome. Clinically significant endogenous viral infections, including EBV and CMV, following AHSCT are increasingly recognized. The incidence of viral reactivation seems to be closely related to the dosage of ATG used in the conditioning regimen(Nash et al., 2003). In the London cohort (Mehra et al., 2019), where 7.5 mg/kg ATG was used, cytomegalovirus reactivation was detected in 26 cases, and preemptive treatment with valganciclovir or ganciclovir was required in 12 of 26 cases, with no CMV disease observed. In a subset of 85 participants with serial EBV DNA copy numbers in blood measurement from the same cohort, EBV reactivation was demonstrated in 80% of participants after AHSCT (20 of whom were treated with rituximab). When ATG was used at a total dose of 5 mg/kg, EBV and CMV reactivation were not reported. Finally, patients undergoing AHSCT are at risk

for malnutrition which is associated with higher risk of infections, delayed neutrophil engraftment and prolonged hospital stay and should be carefully screened for weight loss.

The treatment-associated mortality of AHSCT (defined as mortality within 100 days from AHSCT) has greatly improved in recent years. Mancardi et al. described data from meta-analysis and registry-based studies indicating that the transplant mortality rate was 7.3% between 1995 and 2000, 1.3% between 2001 and 2007 and 0.2% between 2012 and 2017 and thereafter (Mancardi et al., 2018). This reduction has been attributed to better patient selection and the use of intermediate intensity (lympho- and myeloablative) treatment regimens. As noted by Brittain et al. (Brittain et al., 2022), the 0.2% transplant mortality rate of AHSCT in MS compares favorably with the longer-term mortality risks related to use of some high efficacy DMTs such as natalizumab (0.13%) and alemtuzumab (0.18%). While periprocedural burden and potential risks must be considered carefully, AHSCT, unlike most DMTs, is a one-off treatment with no cumulative toxicity or treatment burden. Willison et al. recently reviewed the cause of death of all patients treated with different types of AHSCT conditioning (Willison et al., 2022). Globally, data seem to suggest that intermediate intensity, lymphoablative CY+ATG protocols might offer a better safety profile, even though it is important to note that several factors not directly related to the intensity of the conditioning regimen contribute to the safety of AHSCT, like the monitoring protocol for viral reactivation, the use of prophylactic antibiotics and the fluid management.

No definite data exist on the risk of malignancies after AHSCT. In the Burt et al. cohort of 507 patients who received CY(Burt et al., 2021a), the authors report no incidence of bladder cancer, myelodysplastic syndrome, or leukemia, but describe one case of death secondary to T cell lymphoma 10 years following AHSCT, as well as one death due to colon cancer 3 years following AHSCT. Other authors reported two cases of breast cancer, one case of cervical intraepithelial neoplasia, two cases of myelodysplastic syndrome, an EBV-related post-transplantation lymphoproliferative disorder and one prostate cancer, (Casanova et al., 2017)(Mariottini et al., 2021)(Samijn, 2006)(Fassas et al., 2011). Bigger cohort studies are needed to assess the risk of secondary malignancies after AHSCT for MS.

Development of a new, secondary, autoimmune disease (2ndAD) in patients with a preexisting (primary) autoimmune condition such as MS, has been reported after treatment with AHSCT at rates ranging from 2% to 14%(Burt et al., 2021b; Daikeler et al., 2011). The most common 2ndAD are B-cells mediated autoimmune conditions such as thyroiditis, immune thrombocytopenia, autoimmune hemolytic anemia, and antiphospholipid syndrome. It seems that an imbalance

between B and T lineage regeneration early after AHSCT may underlie the pathogenesis of secondary autoimmunity. The incidence of secondary autoimmune disorders seems to be higher following lymphoablative compared to myeloablative conditioning regimens (Burt et al., 2021b).

*Patient selection and recommendations*

The trials discussed in this chapter provide critical guidance regarding patient selection. Existing data demonstrate that younger age, shorter duration of disease, relapsing–remitting disease course and lower baseline EDSS are associated with better outcomes after AHSCT. Moreover, disability worsening–free survival seems to be higher in patients with lower treatment exposure, suggesting that AHSCT should be considered early in the MS treatment algorithm. The EBMT-Autoimmune Disease Working Party (ADWP) advises that AHSCT may be considered as a "standard of care" treatment for patients with RRMS with high clinical and MRI inflammatory disease activity (>=2 clinical relapses, or one clinical relapse with gadolinium enhancing or new T2 MRI lesions at a separate timepoint, in the previous 12 months) despite the use of one or more lines of approved DMTs (Sharrack et al., 2020). The EBMT-ADWP noted that "evidence best supports treatment in patients who are able to ambulate independently (EDSS 5.5 or less), who are younger than 45 years and have disease duration less than 10 years". Similarly, the American Society for Blood and Bone Marrow Transplantation has recently endorsed AHSCT as a "standard of care [...] for patients with relapsing forms of MS (RRMS or progressive MS with superimposed activity) who have prognostic factors that indicate a high risk of future disability, including ongoing clinical relapse or MRI lesion activity despite treatment with available DMTs, especially if disease activity continues despite treatment with high-efficacy DMTs and/or worsening disability"(Cohen et al., 2019). It is noteworthy that, given the excellent and long-lasting results of AHSCT in treatment-naive MS patients (Das et al., 2021), AHSCT is indicated as a "clinical option" also before failing a full course of DMT, if aggressive characteristics of MS, as early accrual of severe disability and tumultuous CNS inflammation are present (Das et al., 2021). Finally, these recommendations also highlight that only specialist centers should provide AHSCT to people with MS and, whenever possible, these patients should be enrolled in clinical trials.

Although controversies exist, non-randomized studies suggest that AHSCT can slow down neurological deterioration also in active progressive MS (Boffa et al., 2021; Burt et al., 2003; Mariottini et al., 2022, 2021; Muraro et al., 2017). It has been demonstrated that AHSCT is able to reduce CSF markers of axonal damage and neurofilament light chain levels, slow down cognitive

decline and normalize long-term rates of cerebral gray matter and white matter atrophy, which are core pathological features of progressive MS(Lee et al., 2021; Thebault et al., 2019). However, since patients with progressive MS have an increased transplant-mortality-rate compared to younger patients affected by relapsing-remitting MS (Sormani et al., 2017b), caution should be used in referring these patients to AHSCT.

*AHSCT and MRI measures of brain damage*

AHSCT has a dramatic impact on MRI signs of MS inflammatory activity. Studies reporting serial MRI scans after AHSCT uniformly demonstrate an almost complete suppression of new and gadolinium-enhancing MRI lesions (Atkins et al., 2016; Boffa et al., 2021; Mancardi et al., 2001; Nicholas et al., 2021). Despite the profound anti-inflammatory effect of AHSCT, multiple cohorts of MS patients treated with AHSCT have consistently shown accelerated whole-brain volume loss in the first months after treatment (Chen et al., 2006; Lee et al., 2017; Rocca et al., 2007; Roccatagliata et al., 2007). Some studies have suggested that the cytotoxic effect of high-dose chemotherapy used for AHSCT may be one reason for the accelerated brain atrophy (Lee et al., 2018, 2016; Thebault et al., 2020), while pathological processes such as Wallerian degeneration following acute inflammation may contribute as well. Another hypothesis is that early accelerated brain volume loss could be secondary to the so-called pseudoatrophy phenomenon. Pseudo-atrophy has been described following different DMTs in active MS and is thought to be related to the rapid resolution of inflammatory edema, causing shrinkage of MS lesions and changes in white-matter and cortical microstructure (de Stefano and Arnold, 2015). In line with this hypothesis, in some studies the brain volume loss was associated with the extent of gadolinium-enhancing lesions at baseline (Lee et al., 2021; Rocca et al., 2007). However, in the Canadian study, authors did not find evidence for a change in brain water content (Lee et al., 2017b) and found that brain volume loss was associated with the dosage of busulfan, which is known to be highly neurotoxic (Lee et al., 2017a). An important consideration is whether different regimens can be associated with different levels of chemotherapy-related brain volume loss. In BEAM-based AHSCT the rates of brain volume changes were of -1.10/-1.87 %, while studies that used high intensity regimen showed an average brain volume loss of -2.1/-2.3 % following TBI+CY and -3.2% following Busulfan+CY(Lee et al., 2021). While direct comparison of these results is limited because of the different patient populations, MRI protocols and analysis methods, it appears that patients who received BEAM+ATG had relatively less volume changes during early follow-up, as compared to those treated with TBI+CY or

Busulfan+CY. Few studies have investigated the impact of AHSCT on MRI metrics of demyelination and remyelination using quantitative MRI (Brown et al., 2013; Chen et al., 2008). Data from the Canadian cohort show that after AHSCT, most of MS lesions exhibit an improvement in magnetization transfer ratio (MTR), reflecting remyelination, with subjects who experienced clinical improvement showing the most important recovery of MTR values.

## 1.4 Acute and chronic neuroinflammation

Following the acute inflammatory demyelinating phase at lesion onset, each MS lesion may undergo several fates over time, including inflammation resolution, persistence of microglia-induced neuroinflammation, irreversible demyelination or efficient myelin repair. A recent classification of MS lesions, based on the presence/absence and distribution of macrophages/microglia (inflammatory activity) and the presence/absence of ongoing demyelination (demyelinating activity), has been proposed, differentiating between active, mixed active/inactive, and inactive lesions with or without ongoing demyelination (Kuhlmann et al., 2017). Active lesions are characterized by macrophages/microglia throughout the lesion area, whereas mixed active/inactive lesions have a hypocellular lesion center with macrophages/microglia limited to the lesion border. Inactive lesions are almost completely lacking macrophages/microglia. Active and mixed active/inactive lesions can be further subdivided into lesions with ongoing myelin destruction (demyelinating lesions) and lesions in which the destruction of myelin has ceased, but macrophages are still present (post-demyelinating lesions).

In mixed active/inactive lesions, that have been referred to as “chronic active lesions” or “slowly expanding/smoldering lesions”, the persistence of activated microglia can be extensive throughout the lesion or remains confined at the lesion border, with the center of the plaque being hypocellular (Frischer et al., 2015). Most microglia and macrophages found at the rim of chronic active multiple sclerosis lesions contain iron (Hametner et al., 2013), and conversely iron-enriched microglia and macrophages are not found at the rim of remyelinated or inactive plaques (Popescu et al., 2017). The biological reasons underlying persisting innate immune cell activation in MS lesions remains unknown. In their pro-regenerative phenotype, microglia cells could play a beneficial role at the acute/subacute stage lesions, by clearing potentially toxic debris and promoting repair (Lloyd and Miron, 2019). However, as lesions and disease progress, cellular homeostasis is disrupted, and microglia mostly adopt a chronic proinflammatory, degeneration-associated fate.

Disease duration, clinical course, age, and gender contribute to the dynamic nature of white matter MS pathology. Active MS plaques predominate in acute and early RRMS and are the likely substrate of clinical attacks. Progressive MS transitions to an accumulation of smoldering plaques characterized by microglial activation and slow expansion of pre-existing plaques (Frischer et al., 2015).

While gadolinium enhancement serves as a reliable marker of acute, active, lesions, detection *in vivo* of mixed active/inactive lesions confined behind an intact blood-brain-barrier remains challenging. MRI with a gradient echo sequence is sensitive to iron and has been explored by many investigators to detect an iron rim in mixed active/inactive lesions as a paramagnetic rim lesion (PRL) (Absinta et al., 2016, 2013; Bagnato et al., 2011; Dal-Bianco et al., 2021, 2017; Hammond et al., 2008; Maggi et al., 2020). PRLs are detected in about half of MS patients, with a median of 1 PRL per subject, representing 4-6% of total MS lesions. In several independent cohorts, a higher number of PRLs was associated with ongoing degeneration and with a more aggressive disease course (Absinta et al., 2019; Blindenbacher et al., 2020; Hemond et al., 2022; Maggi et al., 2021).

It should be noted that gradient echo, as well as phase contrast sequences, reflect more than just the presence of iron, but rather is affected by a combination of local tissue susceptibility properties that can include loss of diamagnetic myelin, changes in paramagnetic deoxyhemoglobin, and presence of free radicals. Quantitative susceptibility mapping (QSM) provides an effective means to directly map the distribution of susceptibility sources overcoming problems encountered with other gradient echo approaches, like the field-to-source inversion problem and occurrence of blooming artefacts. Thus, QSM has been established as a more sensitive and quantitative technique for measuring brain iron as compared to T2*, R2 and R2* (Deistung et al., 2013). It has been well established that iron and myelin are the main contributors of magnetic susceptibility in white matter. While iron is a paramagnetic material with positive susceptibility with respect to water, myelin is diamagnetic and has negative susceptibility. Accordingly, the susceptibility of a fully demyelinated lesion without any iron accumulation should be at most zero (when the reference susceptibility is CSF), and lesion susceptibility above zero on QSM provides a more specific indication of iron.

A recent combined MRI-histopathological study assessed the specificity of QSM in phenotyping MS lesions according to their degree of inflammation and demyelination (Rahmanzadeh et al., 2022). 71% of QSM homogenously hyperintense lesions in vivo were chronic inactive lesions at post-mortem histopathology, while 21% chronic active. About 95% of PRLs appeared as mixed active/inactive lesions at histology, with iron-laden macrophages-microglia at lesion border. Further 7% chronic active lesions appeared homogenously hyperintense at QSM. Conversely, iso- and hypo-intense lesions exhibited 100% specificity to histopathologically-defined remyelinated lesions.

# 2. THESIS PROJECT

## 2.1 Background and overarching aims

Despite the use of new high efficacy therapies, complete disease remission is elusive in most MS patient. This is particularly relevant for patients affected by aggressive MS, who deal with frequent relapses and accelerated accrual of irreversible disability. No evidence-based criteria exist to guide the choice on the best treatment approach in these patients and recent international guidelines do not address this problem.

It is therefore urgent to collect data on available therapies for aggressive MS, analyzing their onset of action, the intensity of their immunosuppressive effects and their long-term clinical outcomes.

Moreover, given that chronic, smoldering inflammation behind an intact blood-brain barrier has been identified as one of the most important drivers of disability progression, it is of fundamental importance to evaluate the impact of therapies on chronic, smoldering neuroinflammation. This is particularly true for newer treatments, including hematopoietic stem cell transplantation (AHSCT), which are currently under investigation as a treatment strategy for aggressive MS. Quantitative susceptibility mapping (QSM) is a promising advanced MRI technique which allows identification of chronic inflammation within MS lesions. Using QSM it is indeed possible to disentangle MS lesions heterogeneity and to identify paramagnetic rim lesions (PRLs), which have been proposed as a biomarker for mixed active/inactive lesions and are known to correlate with a more aggressive disease course.

Against this background, the overarching aims of this thesis project were to:

1) Assess the long-term outcomes of patients with aggressive MS treated with different highly active immunotherapies
2) Investigate in vivo the impact of different highly active immunotherapies on chronic inflammation within MS lesions

For the first aim of this project, we performed three different separate studies evaluating the impact of (i) AHSCT, (ii) alemtuzumab and (iii) ocrelizumab on clinical and MRI outcomes in patients presenting an aggressive form of MS.

For the second aim, we first performed a longitudinal prospective quantitative MRI study evaluating the impact of AHSCT and other highly active therapies on acute and chronic inflammation in aggressive MS patients. Then, aiming at comprehensively detect the smoldering inflammatory component in MS lesions other than PRLs, we performed a combined quantitative MRI study investigating the relationship between axonal integrity, myelin content and iron deposition in the entire spectrum of MS lesions.

## 2.2 Long-term clinical outcomes of autologous hematopoietic stem cell transplantation in aggressive multiple sclerosis

Abstract

Objective: To determine whether autologous hematopoietic stem cell transplantation (AHSCT) can induce durable disease remission in people with aggressive multiple sclerosis (MS), we analyzed the long-term outcomes after transplant in a large Italian cohort of MS patients.

Methods: To be included, a minimum data set (consisting of age, MS phenotype, EDSS at baseline, information on transplant technology and at least 1 follow-up visit after transplant) was required.

Results: 210 patients were included [relapsing-remitting (RR)MS=122(58%)]. Median baseline EDSS was 6(1-9), mean follow-up was 6.2(±5.0) years. Among RRMS patients, disability worsening-free survival (95%CI) was 85.5% (76.9-94.1%) at 5 years and 71.3% (57.8-84.8%) at 10 years. In patients with progressive MS, disability worsening-free survival was 71.0% (59.4-82.6%) and 57.2% (41.8-72.7%) at 5 and 10 years, respectively. In RRMS patients, EDSS significantly reduced after AHSCT [p=0.001; mean EDSS change per year -0.09 (95%CI=-0.15 to -0.04%)].

Conclusions: AHSCT prevents disability worsening in most patients and induces durable improvement in disability in patients with RRMS. The BEAM+ATG conditioning protocol is associated with a more pronounced suppression of clinical relapses and MRI inflammatory activity.

Classification of Evidence: This study provides Class IV evidence that for people with MS, AHSCT induces durable disease remission in most patients.

*Study design*

This study was an observational, retrospective, multicenter cohort study on AHSCT for the treatment of MS, collecting data from MS patients transplanted in Italy from 1997 to 2019.

In July 1998, five Italian neurologic teams, together with the Italian Cooperative Group for Bone Marrow and Blood Transplantation (GITMO), initiated a phase I/II trial on the use of AHSCT in MS18. Thereafter, other Italian MS centers developed local transplant programs for MS patients, (mostly identical to those developed by the two leading haemato-neurological centers in Italy -Florence and Genoa-). Although no formal guidelines on patients' selection for AHSCT exist, all treated patients had aggressive MS, characterized by the occurrence of severe relapses or MRI inflammatory activity,

or accelerated accrual of neurological disability despite active treatment. Patients were treated with AHSCT according to the European Group for Blood and Marrow Transplantation (EBMT) guidelines (Sharrack et al., 2020), following the decision of the treating physician and approval of the local Ethics Committee. Peripheral blood stem cells were mobilized using cyclophosphamide 4 g/sqm in a single dose followed by filgrastim 10 mcg/Kg/day from day +5 until the completion of stem cells collection. Apheresis begins according to the CD34+ count and continues until a minimum of 8 x 106 CD34+ cells/kg body weight is obtained. Conditioning regimen included carmustine (300 mg/sqm), cytosine-arabinoside (200 mg/sqm), etoposide (200 mg/sqm) and melphalan (140 mg/sqm). The frozen graft containing 3-8 x 106 CD 34+ cells/kg body weight was then thawed and infused iv. Rabbit Anti-T globulin (ATG) was administered at day +1 and +2 at a total dosage of 5 mg/Kg. Anti-viral (valaciclovir 1000 mg po qd or acyclovir 800 mg po bid) prophylaxis was administered during neutropenia and for a minimum of 12 months after discharge and until lymphocytes CD4+ ≥200/mm3. Antibiotic prophylaxis (trimetoprim-sulphametaxol 500 mg po three times a week or pentamidin 300 mg inhalation every 28 days) was administered starting from neutrophils engrafment for a minimum of 12 months after discharge and until lymphocytes CD4+ ≥ 200/mm3.

To be included in the present retrospective study, a minimum data set [consisting of age, MS phenotype, expanded-disability-status-scale (EDSS) at baseline, information on the transplant technology and at least 1 follow-up visit after transplant] was required. For the analysis of MRI disease activity, only patients with yearly brain MRI records were considered.

*Study endpoints*

The primary endpoint was to analyze the long-term 6 months-confirmed disability worsening as measured by EDSS. Secondary objectives were the evaluation of (i) the evolution of the EDSS scores after transplant, (ii) the occurrence of relapses, (iii) the occurrence of MRI inflammatory activity, (iv) the proportion of patients achieving “no-evidence-of-disease-activity (NEDA) status”, a composite endpoint which includes the absence of clinical relapses, EDSS worsening and MRI inflammatory activity. The analysis of the primary and the secondary endpoints generate class IV evidence of the long-term effects of transplant in people with aggressive MS. Disability worsening was defined as an increase of 1 point in the EDSS score (0.5 points if the baseline EDSS score was ≥5.5) confirmed after 6 months. Baseline was defined as the last neurological assessment before the administration

of mobilizing therapy. All relapses were clinically assessed by treating neurologists. Follow-up for any component of NEDA score was not censored by earlier events so that each has an independent interpretation. MRI activity was defined as the presence of new/enlarging T2 lesions or T1 gadolinium-enhancing lesions detected by radiologists on routine follow up MRI. The baseline brain MRI (acquired within 3 months before the AHSCT procedure) was the pre-treatment reference scan for assessment of treatment failure and no re-baseline was performed.

*Statistical analyses*

The probability of disability worsening-free survival, relapse-free survival, MRI-activity free-survival and NEDA-3 status was calculated with the Kaplan-Meier estimator. Univariate and multivariate analyses assessing the association of disease- and treatment-related characteristics with survival endpoints were performed using Cox proportional hazards regression analysis models. Variables significantly associated with each outcome event on univariate analysis were included as covariates in the multivariate model. A linear mixed model with random intercept and random slope was carried out to detect changes in the EDSS scores before vs after transplant. A two-sided $p<0.05$ was used for statistical significance. All analyses were performed using SPSS 23 (IBM; version 23.0) and R software.

*Patients' demographics and procedures*

Patients from 20 Italian MS centers who underwent transplant from 1997 to 2019 were identified (n=210). Demographic, clinical, and hematological characteristics of the study cohort are summarized in Table 1. Out of 210 patients, n=196 (93.3%) were eligible for the analysis of the primary endpoint. As for relapse occurrence, data were available for 198 (94.3%) patients. Serial brain MRI radiology records were available for 167 (79.5%) patients. At the time of transplant, 122 patients (58%) had a relapsing-remitting (RR) phenotype of MS (RRMS), 86 patients (41%) had secondary progressive (SP) MS and 2 patients (1%) had primary-progressive MS.

Table 1. Demographic, disease-related and treatment-related characteristics

| | **Study Cohort (n=210)** | **Relapsing-remitting MS (n=122)** | **Progressive MS (n=88)** |
|---|---|---|---|

| | | BEAM+ATG (n=90) | Other conditioning protocols (n=32) | BEAM+ATG (n=67) | Other conditioning protocols (n=21) |
|---|---|---|---|---|---|
| **Age, mean (SD), y** | 34.8 (8.6) | 34.0 (8.7) | 28.3 (5.7) | 38.0 (7.3) | 37.8 (9.6) |
| **Females, n (%)** | 148 (70.5) | 64 (71.1) | 24 (75.0) | 48 (71.6) | 12 (57.1) |
| **Disease duration, mean (SD), y** | 11.0 (6.7) | 10.3 (6.7) | 7.1 (3.5) | 13.2 (6.7) | 13.2 (7.2) |
| **EDSS, median (IQR)** | 6.0 (4.5-6.5) | 5.0 (3.0-6.0) | 6 (3.0-6.0) | 6.5 (6.0-7.0) | 6.5 (5.5-7.0) |
| **EDSS one year before AHSCT** | | | | | |
| **Median (IQR)** | 5.0 (3.0-6.0) | 4 (2.5-5.5) | 3.5 (2.0-5.0) | 6 (5.0-6.5) | 5.0 (3.5-6.0) |
| ***Missing, n (%)*** | *19 (9.0)* | *11 (12.2)* | *0 (0)* | *4 (6.0)* | *2 (9.5)* |
| **Delta EDSS in the year before AHSCT** | | | | | |
| **Mean (SD)** | 0.8 (1.7) | 0.9 (2.0) | 1.0 (2.1) | 0.6 (0.7) | 0.9 (1.2) |
| ***Missing, n (%)*** | *17 (9.0)* | *11 (12.2)* | *0 (0)* | *4 (6.0)* | *2 (9.5)* |
| **Number of relapses in the year before AHSCT** | | | | | |
| **Mean (SD)** | 1.8 (1.6) | 2.2 (1.6) | 2.5 (1.8) | 1.1 (1.1) | 1.5 (1.7) |
| ***Missing, n (%)*** | *19 (8.1)* | *9 (10.0)* | *2 (6.2)* | *7 (10.4)* | *1 (4.8)* |
| **Number of patients with active MRI scan at baseline** | | | | | |
| **Number (%)** | 112 (73.2) | 37 (75.5) | 19 (73.1) | 30 (85.7) | 11 (57.9) |
| ***Missing, n (%)*** | *57 (27.1)* | *41 (45.6)* | *6 (18.8)* | *32 (47.8)* | *2 (9.5)* |
| **Number of DMTs before AHSCT** | | | | | |
| **Median (IQR)** | 3 (2-4) | 3 (2-4) | 3 (2-4) | 2 (1-3) | 3 (2-4) |
| ***Missing, n (%)*** | *8 (3.8)* | *3 (3.3)* | *0 (0)* | *4 (6.0)* | *1 (4.8)* |
| **Follow-up, mean (SD), y** | 6.2 (5.0) | 5.1 (4.4) | 7.2 (4.6) | 7.6 (5.7) | 5.1 (3.6) |
| **Follow-up, median (IQR), y** | 4.2 (2.1-10.7) | 3.5 (2.1-6.9) | 6.6 (3.0-12.0) | 6.9 (2.3-11.8) | 4.9 (1.6-5.1) |
| **Conditioning regimes, n (%)** | | | | | |
| **BEAM+ATG** | 157 (74.8) | 90 (100) | / | 67 (100) | / |
| **BEAM** | 10 (4.8) | / | 6 (18.8) | / | 4 (19.0) |
| **FEAM** | 4 (1.9) | / | 4 (12.5) | / | 0 (0) |
| **CY+ATG** | 27 (12.9) | / | 15 (46.9) | / | 12 (57.1) |
| **Thiothepa+CY** | 10 (4.8) | / | 6 (18.8) | / | 4 (19.0) |
| **Others** | 2 (1.0) | / | 1 (3.3) | / | 1 (4.8) |

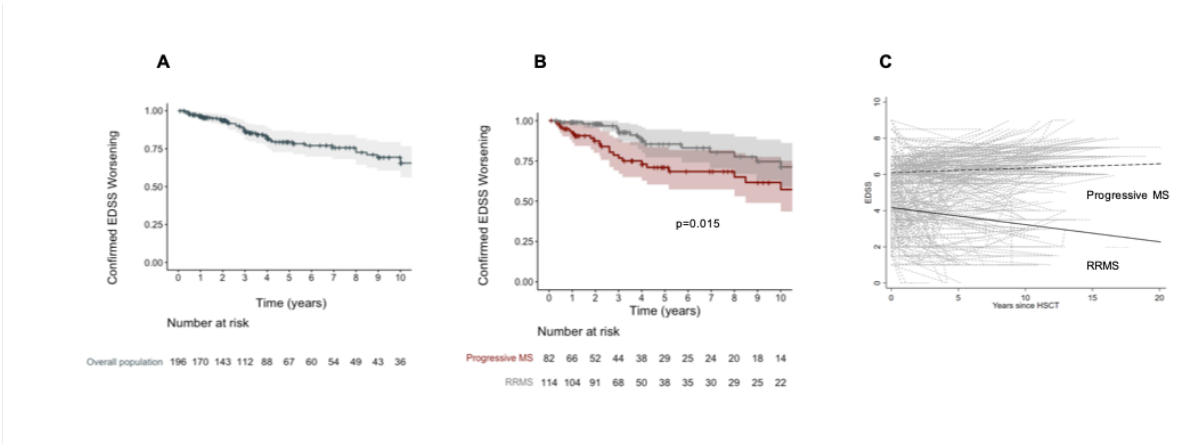
n=number, SD=standard deviation; MS=multiple sclerosis; EDSS=expanded-disability-status-scale; IQR=interquartile range; AHSCT= autologous hematopoietic stem cell transplantation;

MRI=magnetic resonance imaging; DMTs: disease modifying therapies; BEAM=conditioning regimen composed of carmustine, etoposide, cytarabine, melphalan; ATG= rabbit anti-thymocyte globulin; FEAM=conditioning regimen composed of fotemustine, etoposide, cytarabine, melphalan; CY=cyclophosphamide

*Disability worsening-free survival and the evolution of neurological disability*

The probabilities of disability-worsening free survival for the entire study cohort and according to disease phenotype are reported in Figure 1A and 1B, respectively. In the entire study cohort, disability worsening-free survival was 79.5% (72.0-86.6%) and 65.5% (55.3%-75.7%) at 5 and 10 years. The RRMS phenotype was associated with a reduced risk of disability worsening [HR (95%CI)= 0.46 (0.24-0.86), p=0.015], with disability worsening-free survival rates of 85.5% (76.9%-94.1%) at 5 years and 71.3% (57.8%-84.8%) at 10 years. In RRMS, a higher treatment exposure before AHSCT was associated with a higher risk of disability worsening [HR=1.57 (1.12-2.20), p=0.009]. Among patients with progressive MS, disability worsening-free survival was 71.0% (59.4%-82.6%) and 57.2% (41.8%-72.7%) at 5 and 10 years, respectively.

Figure 1. Disability worsening-free survival and the evolution of the neurological disability.
Panel A shows the probabilities of disability worsening-free survival after AHSCT for the entire study cohort. Panel B shows disability worsening-free survival curves according to the MS phenotype. Panel C shows the evolution of the neurological disability in patients with RRMS and with progressive MS.



EDSS= expanded disability status scale; MS= multiple sclerosis; RRMS= relapsing-remitting multiple sclerosis.

A higher number of relapses in the year before AHSCT was associated with a lower risk of disability worsening [HR=0.56 (0.34-0.92), p=0.022]. The use of the BEAM+ATG conditioning protocol did not influence the probabilities of disability worsening free survivals. Progression-free survival in RRMS patients who were transplanted with the BEAM+ATG protocol was 81.9% (70.1%-93.7%) at 5 and 10 years. Figure 1C shows the evolution of EDSS scores recorded after AHSCT in patients with RRMS and progressive MS. Among patients with RRMS, median EDSS scores significantly reduced after transplant over 10 years [p=0.001, mean EDSS change per year -0.09 (95%CI= -0.15 to -0.04)]. EDSS stabilized in patients with progressive MS, with no significant increase over time [p=0.42, mean EDSS change per year=0.02 (95%CI= -0.03 to 0.07)].

*Secondary endpoints*

The probabilities of relapse-free survival, MRI inflammatory activity-free survival and NEDA-3 status are reported in Figure 2 (RRMS) and Figure 3 (progressive MS), according to the conditioning regimen used in the transplant technology. For RRMS patients, relapse-free survival was 78.1% (68.5%-87.7%) and 63.5% (49.4%-77.6%) at 5 and 10 years after AHSCT. In RRMS patients treated with the BEAM+ATG protocol, relapse-free survival was 86.4% (75.8%-97.0%) and 77.0% (61.5%-92.5%) at 5 and 10 years. The use of the BEAM+ATG conditioning protocol [HR= 0.21 (0.09-0.49), p<0.0001] and an older age at transplant [HR=0.94 (0.88-0.99), p=0.034] were independently associated with a reduced risk of relapses. Among patients with progressive MS, relapse-free survival was 88.3% (80.7%-96.0%) and 78.9% (63.4%-91.4%) at 5 and 10 years, respectively. The use of the BEAM+ATG conditioning protocol [HR=0.25 (0.71-0.86), p=0.029] was associated with a reduced risk of a relapse. In the entire study cohort, relapse-free survival was 82.9% (76.6%-89.2%) and 71.2% (61.8%-80.6%) 5 and 10 years after AHSCT, respectively.

Figure 2. Relapse-free survival, MRI inflammatory activity-free survival and No Evidence of Disease Activity (NEDA-3) status in patients with RRMS.

Panels 2A, 2C and 2E show the probabilities of relapse-free survival, MRI inflammatory activity-free survival and NEDA-3 percentages for patients with relapsing-remitting MS. Panel 2B, 2D and 2F show the survival curves according to the conditioning regimen used within the transplant technology.

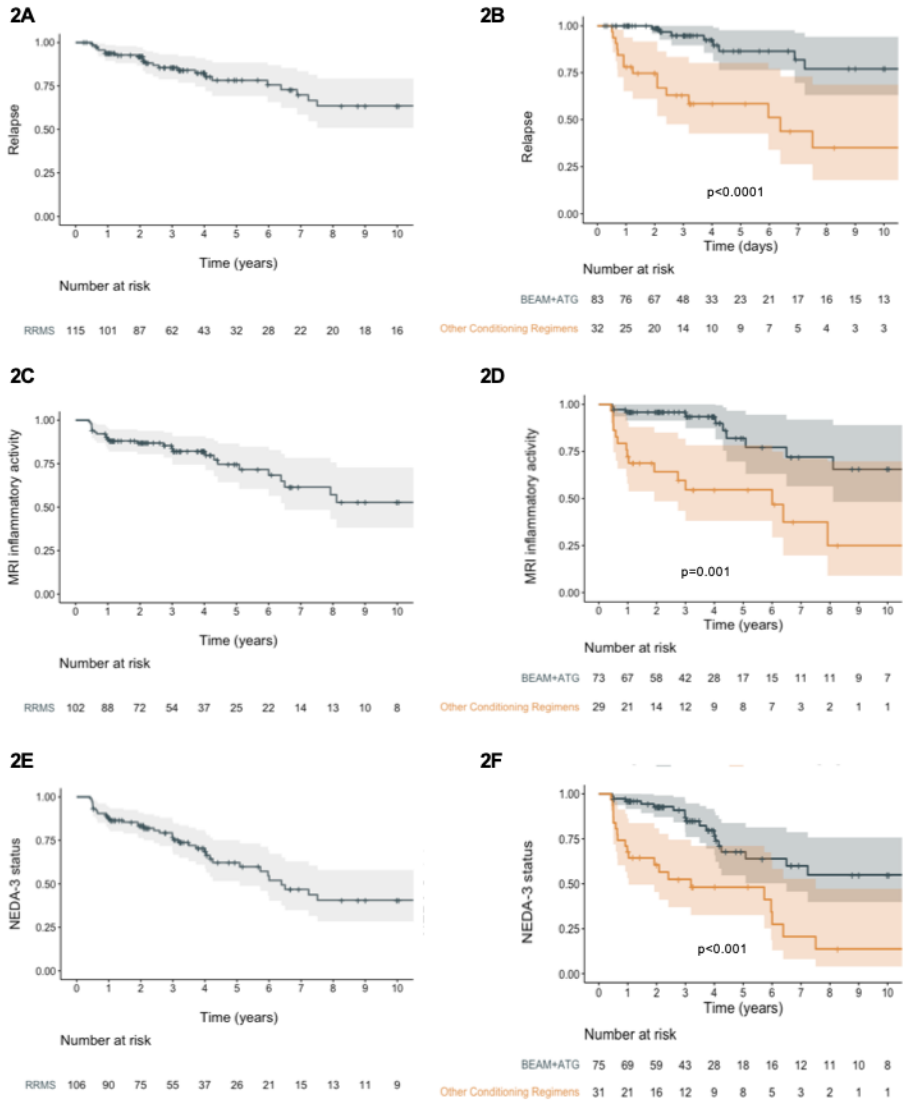
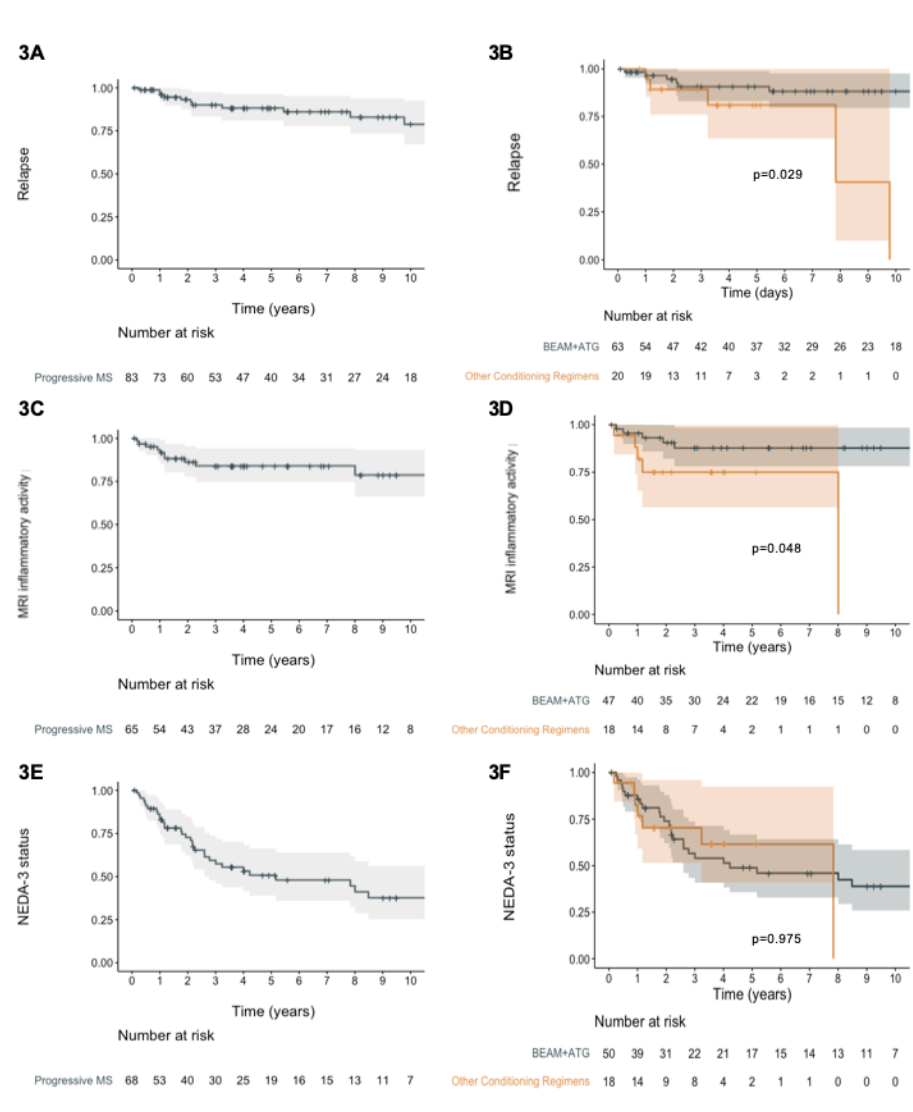


Figure 3. Relapse-free survival, MRI inflammatory activity-free survival and No Evidence of Disease Activity (NEDA-3) status in patients with progressive MS.

Panels 3A, 3C and 3E show the probabilities of relapse-free survival, MRI inflammatory activity-free survival and NEDA-3 percentages for patients with progressive MS. Panel 3B, 3D and 3F show the survival curves according to the conditioning regimen used within the transplant technology.




Probabilities for MRI inflammatory activity-free survival for patients with RRMS were 74.6% (63.2%-85.6%) at 5 years and 52.7% (35.6%-69.7%) after 10 years. When the BEAM+ATG was used, the MRI inflammatory activity-free survival was 82.0% (68.5%-95.5%) and 65.5% (45.3%-85.7%) at 5 and 10 years, respectively. The use of the BEAM+ATG conditioning regimen [HR=0.24 (0.11-0.54), p=0.001] and an older age [HR=0.93 (0.88-1.00), p=0.041] were independently associated with a reduced risk

of MRI inflammatory activity after AHSCT. In the subgroup of patients with progressive MS, the MRI inflammatory activity-free survival was at 84.0% (74.2%-93.8%) and 78.7% (65.2%-92.2%) at 5 and 10 years, respectively. The use of the BEAM+ATG protocol was found to be associated with a higher probability of suppression of MRI inflammatory activity [HR=0.28 (0.08-1.00), p=0.048]. In the entire study cohort, the percentages of patients free of MRI inflammatory activity were 78.7% (71.1%-86.3%) at 5 years and 64.3% (52.7%-75.9%) at 10 years.

For patients with RRMS, probabilities of achieving NEDA-3 status were 62.2% (50.6%-73.8%) at 5 years and 40.5% (30.0%-55.0%) at 10 years. In the subgroup of RRMS patients who underwent AHSCT with the BEAM+ATG conditioning protocol, NEDA-3 status was achieved in 67.7% (53.2%-82.2%) and 54.9% (37.3%-72.5%) of patients at 5 and 10 years, respectively. In RRMS patients, the use of the BEAM+ATG protocol [HR=0.27 (0.14-0.50), p<0.001] was associated with a higher probability of maintaining NEDA-3 status. In patients with progressive MS, NEDA-3 status estimates were 50.8% (37.3%-64.3%) and 37.3% (22.8%-52.6%) at 5 and 10 years respectively, and no baseline characteristics were found to be associated with the probability of NEDA-3 status. In the entire study cohort, NEDA-3 status was achieved in 57.9% of patients (49.1%-66.7%) at 5 years and in 39.8% of patients (29.2%-50.4%) 10 years after AHSCT.

When comparing the BEAM+ATG conditioning regimen with the cyclophosphamide-based protocols alone, we confirmed that, in patients with RRMS, the use of the BEAM+ATG was associated with a lower risk of relapse [HR=0.12 (0.05-0.32), p<0.001], MRI inflammatory activity [HR=0.18 (0.07-0.48), p=0.001] and with a higher probability of maintaining NEDA-3 status [HR=0.18 (0.09-0.38), p<0.001] over the entire follow-up. In patients with progressive MS, we did not find any difference between BEAM+ATG and cyclophosphamide-based regimens on treatment response.

*Discussion*

Multiple sclerosis-related disability might take many years or decades to develop, and very long follow-up periods are required to understand the role of treatments for MS.

We herein report the long-term outcomes in a large cohort of aggressive MS patients who underwent AHSCT in Italy in the last two decades, showing that 65.5% of patients were free of disability worsening 10 years after transplant, with a disability worsening-free survival greater than 70% in patients with RRMS. In line with previous observations (Muraro et al., 2017), disability

worsening-free survival in our cohort was higher in RRMS patients with lower treatment exposure, confirming the notion that AHSCT should be performed early in the course of the disease.
In this study, we had the opportunity to analyze serial MRI records from 167 patients. Available long-term longitudinal MRI data after AHSCT are scarce and limited by small sample sizes. In our cohort of patients with RRMS treated with BEAM + ATG, 65.5% of patients were free of MRI inflammatory activity at 10 years. These results are impressive, considering that MRI activity is seen in 50% to 60% of patients treated with alemtuzumab and ocrelizumab in a typical 2-year follow-up (Sormani et al., 2017a). Similarly, the percentages of NEDA-3 status at 5 and 10 years in the subgroup of patients with RRMS treated with BEAM + ATG (67.7% and 54.9%, respectively) are higher than those reported in randomized clinical trials for available therapies (Sormani et al., 2017a). However, these data should be interpreted with caution because patient populations and the follow-up schedules, as well as the use of a re-baseline MRI scan for MRI activity assessment, differ greatly between clinical studies.

The optimal intensity of the conditioning regimen for the treatment of MS remains an open question. This is the first study suggesting that the use of the BEAM+ATG conditioning regimen is independently associated with a reduced probability of relapses, MRI activity and NEDA-3 failure in patients with RRMS. Our results are in line with the evidence that a high-intensity, busulfan-based (Atkins et al., 2016), but not a low-intensity cyclophosphamide-based (Curro et al., 2015), conditioning regimen was able to completely abrogate MRI activity and clinical relapses. These results are also in line with the evidence that the bone marrow is the major site of memory helper T cells and memory plasma cells which are resistant to treatment with cyclophosphamide and that could be responsible for the maintenance of the autoimmune process over time(Mumtaz et al., 2012). However, our results should be interpreted with caution because of the relatively small number of patients transplanted with cyclophosphamide-based regimens. Moreover, the cyclophosphamide protocols analyzed in this study are slightly different to the one used by Burt and colleagues(Burt et al., 2019a), preventing direct comparisons.

*Limitations*

Our work suffers from several methodological limitations. First, the EDSS raters were not blinded to treatment, and this could have introduced some bias. However, the long-term design of this study

has partially mitigated this measurement bias. Second, we had no information about the time between last clinical relapse and transplant start and we could not correct for this confounder when analyzing EDSS improvement over time, that can be thus overestimated. Third, clinical and MRI assessments were not systematically performed throughout the study. To overcome this bias, only patients with 6-months confirmed EDSS assessment and yearly MRI records were included in the analysis of treatment effects.

## 2.3 Aggressive multiple sclerosis: treatment experience with autologous hematopoietic stem cell transplantation and alemtuzumab

Abstract

Background and purpose: The best therapeutic approach for aggressive relapsing–remitting multiple sclerosis remains unknown. The objective was to compare the efficacy and safety of autologous hematopoietic stem cell transplantation (AHSCT) and alemtuzumab in aggressive relapsing–remitting multiple sclerosis.

Methods: The time to first relapse, time to confirmed disability worsening, time to first evidence of magnetic resonance imaging (MRI) activity and time to first evidence of disease activity were compared between the two treatment groups. Secondary outcomes included the 12-, 24- and 36-month annualized relapse rate (ARR) and the 6-month confirmed Expanded Disability Status Scale (EDSS) changes at months 12 and 24.

Results: Fifty-seven patients treated with AHSCT (n = 25) or alemtuzumab (n = 32) were included. At baseline, AHSCT patients had a higher EDSS (median score 6 vs. 3; P < 0.001), higher ARR (mean ARR 3.2 vs. 1.7; P = 0.001) and a higher number of baseline T1 gadolinium-enhancing lesions on MRI (mean number 15.5 vs. 1.6; P < 0.001). NEDA-3 (no evidence of disease activity) status was more frequently achieved in AHSCT-treated patients than in alemtuzumab-treated patients [75% vs. 56% of patients at the end of the observation period; hazard ratio (HR) 0.27, 95% confidence interval (CI) 0.08–0.84; P = 0.023]. AHSCT significantly reduced the risk of relapse (relapse-free survival 84% vs. 69%; HR 0.13, 95% CI 0.02–0.63; P = 0.012) and MRI activity (MRI-activity-free survival 85% vs. 59%; HR 0.13, 95% CI 0.03–0.59; P = 0.009). The ARR at 36 months was significantly lower in the AHSCT group (0.05 vs. 0.35, P = 0.02). A significant effect of AHSCT in promoting EDSS improvement compared with alemtuzumab was noted (P = 0.035).

Conclusions: Alemtuzumab and AHSCT are effective treatment choices for aggressive multiple sclerosis. AHSCT seems to be superior to alemtuzumab in inducing complete disease control and in promoting short-term disability improvement.

*Study design*

According to Rush et al. (Rush et al., 2015), patients were defined as having aggressive RRMS if one or more of the following were present: (i) multiple (≥2) relapses with incomplete resolution in the past year; (ii) >2 magnetic resonance imaging (MRI) scans showing new or enlarging T2 lesions or gadolinium-enhancing lesions despite active treatment; (iii) Expanded Disability Status Scale (EDSS) score ≥ 4 within 5 years of onset; and (iv) no response to therapy with one or more active disease-modifying treatments (DMTs) for up to 1 year. All consecutive aggressive RRMS patients treated with alemtuzumab or with a myeloablative AHSCT protocol were eligible for this study. Patients were further screened for the presence of an early secondary progressive (SP) phase of MS, according to the criteria proposed by Lorscheider et al. (Lorscheider et al., 2016)[which consists of disability progression by one EDSS step (0.5 in patients with EDSS ≥ 6) in the absence of a relapse and a minimum EDSS score of 4 and pyramidal functional score of 2 with confirmed progression over ≥3 months, including confirmation within the leading functional score]. Patients meeting Lorscheider criteria for SPMS were excluded from the study. All patients provided informed consent to use their medical history for publication. This study was approved by the local ethical committee.

*Study endpoints*

The primary objectives were to determine and compare time to first relapse, time to confirmed disability worsening, time to first evidence of MRI activity and time to first evidence of disease activity (according to the NEDA-3 definition) in patients treated with AHSCT and in those treated with alemtuzumab. The secondary objectives were to assess:

- the annualized relapse rate (ARR) at 12, 24 and 36 months
- the 6-month confirmed EDSS changes at 12 and 24 months

*Intervention*

AHSCT was performed as described in the previous chapter. Alemtuzumab treatment was administered as scheduled, with the first course consisting of 12 mg/day on 5 consecutive days and the second of 12 mg/day on 3 consecutive days, 12 months apart. Premedication treatment included paracetamol, methylprednisolone (1 g iv) and chlorpheniramine hydrochloride. From the

first day of treatment, all patients started prophylaxis with valacyclovir (500 mg/day for 1 month) and sulfamethoxazole/trimethoprim (800 + 160 mg 3 times/week, associated with folic acid). In two patients, where allergy to sulfonamides was reported, antibacterial prophylaxis was not performed. As for AHSCT patients, antibacterial prophylaxis was discontinued when the T CD4+ lymphocyte count reached values > 200/mm3. Diet recommendations for listeria prevention were given to all patients in both treatment groups.

*Statistical analysis*

The probability of disability-progression-free survival, relapse-free survival, MRI-activity-free survival and NEDA-3 status was calculated with the Kaplan–Meier estimator. Cox proportional hazard models were used to compare time to event differences between the two treatment groups. Confirmed disability progression was defined as an increase of 1 point in the EDSS score (0.5 points if the baseline EDSS score was ≥ 5.5) confirmed after 6 months. The baseline brain MRI was the pre-treatment reference scan for assessment of treatment failure. Patients were defined as presenting NEDA over a given period of time if they did not experience any clinical relapse, disability progression or any MRI activity over that period. Follow-up for any component was not censored by earlier events so that each has an interpretation independent of the other components. Five AHSCT-treated patients did not have regular serial brain MRI scan and were therefore excluded from the MRI activity and the NEDA-3 probability analyses. The baseline brain MRI (acquired within 3 months before AHSCT mobilization or alemtuzumab start) was the pre-treatment reference scan for assessment of treatment failure and no re-baseline was performed. ARRs were compared over 12, 24 and 36 months between the two treatment groups using multivariate analysis, accounting for the effects of age, sex, baseline ARR and baseline EDSS. Repeated measures ANCOVA, accounting for age and sex, was carried out in order to detect the interaction treatment 9 time on EDSS changes over 24 months. Baseline differences between the two groups were assessed using Fisher's exact test, the independent samples t test or the Mann–Whitney U test as appropriate. A two-sided $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 23 (IBM; version 23.0; Armonk, NY).

*Study population*

Eighty-five consecutive patients treated either with AHSCT (n = 52) or alemtuzumab (n = 33) at our institution were screened. Twenty-seven AHSCT patients and one alemtuzumab patient were excluded from the study because of the presence of the secondary progressive phase of MS, according to Lublin criteria(Lublin et al., 2014) and Lorscheider criteria for SPMS. All patients with RRMS met the criteria for aggressive MS according to Rush et al. The final study cohort included 25 AHSCT- and 32 alemtuzumab-treated RRMS patients. Demographic and clinical characteristics are reported

in Table 1. At baseline, AHSCT patients had higher EDSS (median EDSS of 6 vs. 3; p< 0.001), higher ARR (3.2 vs. 1.7; p= 0.001) and had more frequently an active baseline MRI scan (88% vs. 43.8%; p< 0.0001) with a higher number of baseline T1 gadolinium-enhancing lesions (mean number 15.5 vs. 1.6; P < 0.001). Mean follow-up was 50.9 (48.2) months for AHSCT-treated patients and 29.3 ( 11.3) months for alemtuzumab-treated patients (p= 0.039).

Table 1. Baseline demographics and clinical features

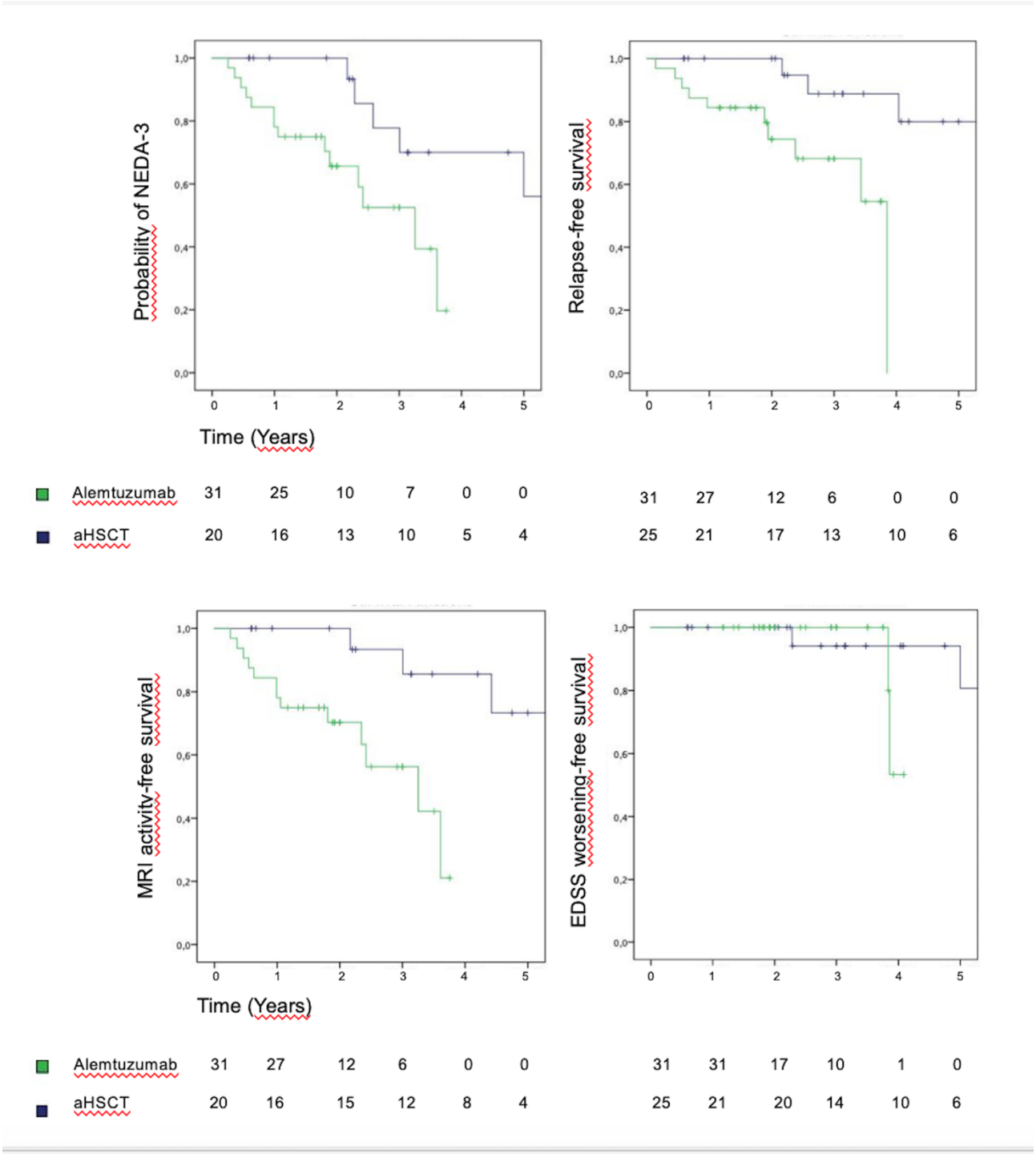
| | **AHSCT (25)** | **Alemtuzumab (32)** |
|---|---|---|
| Females, n (%) | 19 (76) | 24 (75) |
| Mean (±SD) age, y | 32.1 (9.9) | 35.1 (8) |
| Median (IQR) EDSS | 6 (4.5-7) | 3 (1-4) |
| Median (IQR) EDSS -1 year | 4 (3-6) | 2 (1-3) |
| Median (IQR) number of previous DMTs | 3 (2-4) | 2 (1-3) |
| Mean (±SD) disease duration, y | 9.5 (5.4) | 7.2 (5.9) |
| Mean (±SD) ARR | 3.2 (1.7) | 1.7 (1.6) |
| MRI activity at baseline, n (%) | 22 (88) | 14 (44) |
| Mean (±SD) number of Gd+ lesions | 15.5 (28.9) | 1.6 (2.7) |
| Mean (±SD) follow-up, m | 50.9 (48.2) | 29.3 (11.3) |
| Previous treatment exposure, n (%) | | |
| Naive Patients | 0 (0) | 4 (12) |
| Interferon | 17 (68) | 21 (66) |
| Glatiramer Acetate | 5 (20) | 5 (16) |
| Fingolimod | 11 (44) | 20 (62) |
| Natalizumab | 15 (60) | 12 (37) |
| Dimethyl-fumarate | 1 (4) | 7 (22) |
| Mitoxantrone | 4 (16) | 0 (0.0) |

| | | |
|---|---|---|
| Cyclophosphamide | 8 (32) | 4 (12) |
| Azatioprine | 4 (16) | 1 (3) |

Time to first relapse, time to disability worsening, time to first evidence of MRI and overall disease activity

The analysis of the primary end-point (Fig. 1) showed that NEDA-3 status was more frequently achieved in AHSCT-treated patients than in alemtuzumab-treated patients [75% vs. 56% at the end of the observation period; hazard ratio (HR) 0.27, 95% confidence interval (CI) 0.08–0.84; p= 0.023]. AHSCT significantly reduced the risk of relapse (relapse-free survival 84% vs. 69%; HR 0.13, 95% CI 0.02–0.63; p= 0.012) and MRI activity (MRI-activity-free survival 85% vs. 59%; HR 0.13, 95% CI 0.03–0.59; p= 0.009). No differences were noted in the proportion of patients with confirmed disability progression between the two groups (88% vs. 94%; HR 0.25, 95% CI 0.02–2.86; p= 0.263). A 12-month MRI re-baseline was performed, in order to assess the effect of a complete cycle of alemtuzumab on MRI activity. The risk of MRI inflammatory activity remained significantly higher for alemtuzumab-treated patients compared to transplanted ones (MRI-activity free survival 75% vs. 85%; HR 0.15, 95% CI 0.03–0.79; p= 0.025; data not shown). Amongst AHSCT patients who lost NEDA-3 status, three patients had MRI activity and two patients experienced neurological worsening. In the alemtuzumab group, reasons for NEDA-3 failure were MRI activity in four patients and relapses together with MRI activity in 10 patients. Six AHSCT-treated patients started a new DMT (n = 3 dimethyl fumarate, n = 2 ocrelizumab and n = 1 glatiramer acetate) a mean time of 42.5 (14.8) months after transplant. Nine alemtuzumab-treated patients started ocrelizumab a mean time of 22.5 (13.1) months after the second course of alemtuzumab. No patient received more than two alemtuzumab courses. Time to re-treatment was significantly prolonged in the AHSCT group (P = 0.026).

Figure 1 Time to first relapse, time to disability worsening, time to first evidence of MRI activity and NEDA-3 probability.



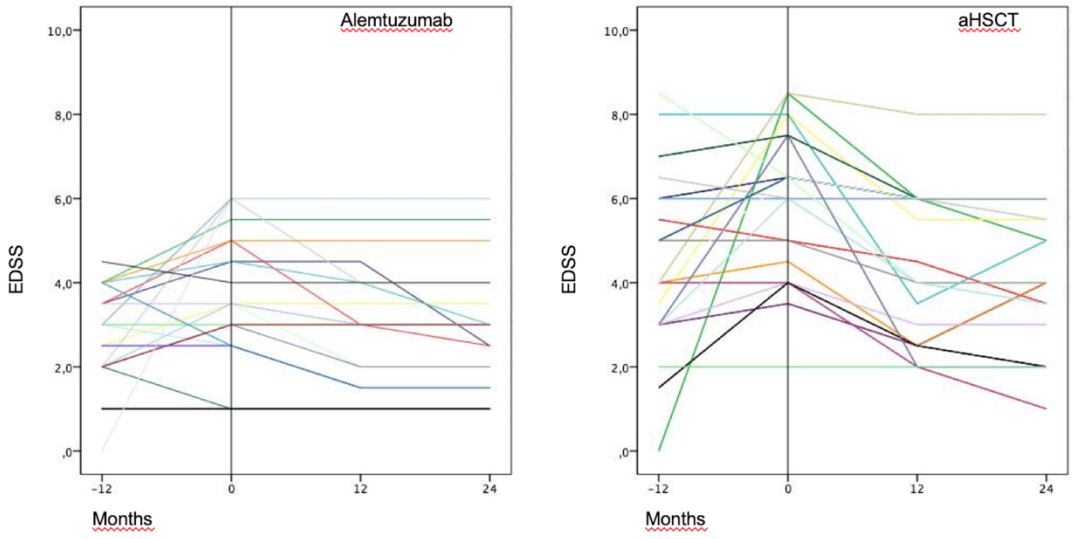
*Relapses*

On multivariate analysis accounting for the effects of sex, age, baseline ARR and baseline EDSS, the ARR at 12 and 36 months was significantly lower in AHSCT-treated patients (0.0 vs. 0.17, p= 0.03, and 0.05 vs. 0.35, p=0.02, respectively). The 24-month ARR did not differ between the two treatment groups (0.1 vs. 0.09). Six out of 15 relapses within the alemtuzumab group occurred between the first and the second treatment course, whilst nine developed after the second course. Amongst the eight patients who experienced relapses after the second alemtuzumab course, four had already experienced relapses between the first and the second treatment course and two had developed asymptomatic MRI evidence of disease activity.

*Disability*

Both AHSCT-treated patients and alemtuzumab-treated patients experienced significant EDSS improvement during the first year after transplant (P < 0.001 and P = 0.001 respectively) (Fig. 2). The interaction treatment 9 time in repeated measures ANCOVA, accounting for the effects of age and sex, showed a significant effect of AHSCT in promoting EDSS improvement compared with alemtuzumab (P = 0.035).

Figure 2. The evolution of neurological disability after treatment start



*Discussion*

No evidence-based criteria exist to guide the choice on the correct treatment for aggressive MS. To the best of our knowledge, this is the first real-world study to assess the efficacy of AHSCT and alemtuzumab in a cohort of aggressive RRMS patients treated in a single MS center. Although the patients allocated in the AHSCT group had significantly higher baseline EDSS scores, higher ARRs and more pronounced MRI activity, AHSCT significantly reduced the risk of relapses and MRI activity, allowing NEDA-3 status in a higher proportion of patients compared with alemtuzumab. No differences were noted in time to EDSS progression between the two treatment groups: this finding could be partly due to the shorter follow-up and to the relative milder disease in alemtuzumab-treated patients. Interestingly, previous studies(Burt et al., 2015; Nash et al., 2017) were confirmed showing that transplanted patients experienced sustained EDSS improvement after treatment, which was more pronounced compared with that seen in alemtuzumab-treated patients. Accordingly, a few patients started a new DMT after transplant and complete disease control was achieved in all cases, even when low-efficacy DMTs were used. Whether this is due to a stronger treatment response following immune resetting after transplant needs to be addressed in further studies. On the other hand, about one-third of alemtuzumab patients were switched to ocrelizumab at a mean time of 1.8 years after the second alemtuzumab course. Although additional alemtuzumab courses seem to improve outcomes (Havrdova et al., 2015), it was decided to switch our alemtuzumab-refractory patients to ocrelizumab because of the presence of B cell-mediated secondary autoimmune disorders, the occurrence of concomitant subtle disability progression and/or the occurrence of inflammatory disease activity after both alemtuzumab courses. Although data from clinical trials suggest that patients with early relapses after the first alemtuzumab course still benefit from re-treatment (van Wijmeersch et al., 2020), no such beneficial effect was observed in our alemtuzumab-treated patients. Accordingly, the risk of MRI inflammatory activity remained significantly higher for alemtuzumab-treated patients compared to transplanted ones even after a 12-month MRI re-baseline.

*Limitations*

This study has several limitations. First, this was a retrospective study and the two study populations were quite unbalanced in terms of age, EDSS score, ARR and MRI activity at the baseline evaluation.

This is not surprising, considering that AHSCT is an off-label treatment in Italy, and it has been reserved to the most aggressive MS patients. Despite the significantly higher disease severity, the MS group treated with AHSCT had a more favorable short-term outcome. Secondly, the clinical assessment was not blinded, and this might have introduced some bias. However, the use of the 6-month confirmed EDSS score as a marker of disability worsening and the use of MRI in the case of suspected relapse has mitigated this bias. In addition, the single-centre design of the study has minimized the inter-rater variability in evaluating efficacy and safety issues.

## 2.4 Predictors of Ocrelizumab Effectiveness in Patients with Multiple Sclerosis

Abstract

Background and aims: Data regarding effectiveness and safety of ocrelizumab in the post-marking setting are lacking. The aim of our study was to provide effectiveness and safety data of ocrelizumab treatment in patients with relapsing–remitting (RR-) and progressive multiple sclerosis (PMS) and to evaluate clinical and immunological predictors of early treatment response, with a special focus on patients with aggressive MS.

Methods: In this single center prospective observational study, we investigated effectiveness outcomes (time-to-confirmed disability worsening, time-to-first relapse, time-to-first evidence of MRI activity and time-to-first evidence of disease activity), clinical and immunological predictors of early treatment response, and incidence of adverse events (AEs).

Results: One hundred and fifty-three subjects were included (93 RRMS; 84 females). Median follow-up was 1.9 (1.3–2.7). At 2-year follow-up (FU), disability worsening free survival were 90.5%, 64.7%, and 68.8% for RRMS, primary-progressive MS (PPMS), and secondary-progressive MS (SPMS) patients, respectively. At 2-year FU, 67.1%, 72.7%, and 81.3% of patients with RRMS, PPMS, and SPMS were free of MRI activity, with NEDA-3 percentages of 62.1%, 54.6%, and 55.1%, respectively. Lower baseline EDSS was independently associated with a reduced risk of disability worsening (HR (95%CI) = 1.45 (1.05–2.00), p = 0.024) and previous treatment exposure was independently associated with increased probability of radiological activity (HR = 2.53(1.05–6.10), p = 0.039). Patients with highly active MS had a higher risk of persistent MRI inflammatory activity (MRI-free survival 58.3% vs 75.5%, HR = 0.35 (0.15–0.82), p = 0.015) and tended to have lower NEDA-3 rates (NEDA-3 percentage of 38.9% vs 60.1, HR = 0.48 (0.22–1.02), p = 0.056) compared to patients without the same baseline characteristics.

Conclusions: Our findings suggest that ocrelizumab is an effective treatment in real-world patients with RRMS and PMS, with a manageable safety profile. Better outcomes were observed in treatment naïve patients and in patients with a low baseline disability level, while patients failing a previous therapies had worse outcomes. Depletion of CD8 + cells could underlie early therapeutic effects of ocrelizumab.

*Study design*

This is an observational prospective single-center cohort study conducted at the MS Center of the University of Genoa, Policlinico San Martino, evaluating effectiveness and safety of ocrelizumab therapy for the treatment of RRMS and PMS. All participants provided consent to use their medical history for publication. Ocrelizumab was prescribed and administered by the treating physician to relapsing forms of MS and early PPMS according to regulatory policies. From March to June 2020, due to coronavirus disease 2019 (COVID19) pandemic, ocrelizumab was administered following a tailored approach evaluating the profile risk of each patient (Zecca et al., 2019), according to international indications (Brownlee et al., 2020). All patients were clinically evaluated every 3 months for assessment of effectiveness and safety. One-hundred forty-five patients (95%) underwent baseline and follow-up MRI scans at our institution with a standardized 3-T MRI protocol (Siemens PRISMA). A subset of patients (n = 73) underwent serial blood samples at our institution every 6 months, before ocrelizumab infusion, for lymphocyte profiling and immunoglobulin concentration.

*Study endpoints*

The primary objective of our study was to determine time-to-first relapse, time-to-confirmed disability worsening, time-to-first evidence of MRI activity, and time-to-first evidence of disease activity (according to the NEDA-3 definition) in real-life patients treated with ocrelizumab. Secondary outcomes were to (i) assess efficacy outcomes in patients with recent high disease activity and those with a relatively high disability at ocrelizumab commencement, (ii) assess clinical and immunological predictors of early inflammatory activity based on flow-cytometry immune subsets characterization during ocrelizumab therapy, and (iii) assess safety of treatment. Baseline brain MRI (acquired within 3 months before ocrelizumab start) was the pre-treatment reference scan for assessment of treatment failure. As exploratory analyses, re-baseline of MRI activity was performed 100 and 180 days after ocrelizumab start. Recent highly active MS was defined, based on the criteria proposed by Rush et al. (Rush et al., 2015), as the presence of MRI activity and at least one relapse in the year before ocrelizumab start in patients with accelerated accrual of disability (EDSS ≥ 4.0). Early inflammatory activity was defined as the occurrence of MRI inflammatory activity or a relapse within the first 12 months of treatment. All the analyses were

performed on the global cohort of patients and, as sensitivity analyses, in the cohort of patients with RRMS and progressive MS separately.

*Statistical analyses*

Descriptive results were reported as mean with standard deviation (SD) or median with interquartile range (IQR). The probability of disability worsening-free survival, relapse-free-survival, MRI activity-free survival, and NEDA-3 status was calculated with the Kaplan–Meier estimator. Univariate and multivariate analyses assessing the association of demographic- and disease-related characteristics with survival endpoints were performed using Cox proportional hazards regression analysis models. Variables significantly associated with each outcome event on univariate analysis were included as covariates in the multivariate model. Differences in lymphocyte subpopulations at different timepoints were assessed with analysis of covariance, adjusting for age, sex, MS phenotype, and last DMT before ocrelizumab initiation. Correction was made for multiple comparisons (Bonferroni-p = 0.0028). Univariate and multivariate binary logistic regression analyses were used to explore the predictive role of clinical and immunological variables in terms of early inflammatory activity. A time*early treatment response group interaction was included into a linear mixed model with random intercept and random slope to test differences on lymphocyte subset values time trend between patients with and without early inflammatory activity. A two-sided $p < 0.05$ was used for statistical significance. SPSS 23 (IBM; version 23.0) and R software (version 4.0.3) were used for computation.

*Study population*

One hundred and fifty-three consecutive MS patients (93 RRMS, 43 PPMS and 17 relapsing, secondary-progressive (SP) MS) initiated treatment with ocrelizumab at the MS Center of the University of Genoa, Policlinico IRCCS San Martino, from July 2017 to July 2020. Demographic- and disease-related characteristics at ocrelizumab start are reported in Table 1.

Table 1. Demographic and clinical characteristics

| | **Total Cohort** (n=153) | **RRMS** (n=93) | **Progressive-MS** |
|---|---|---|---|

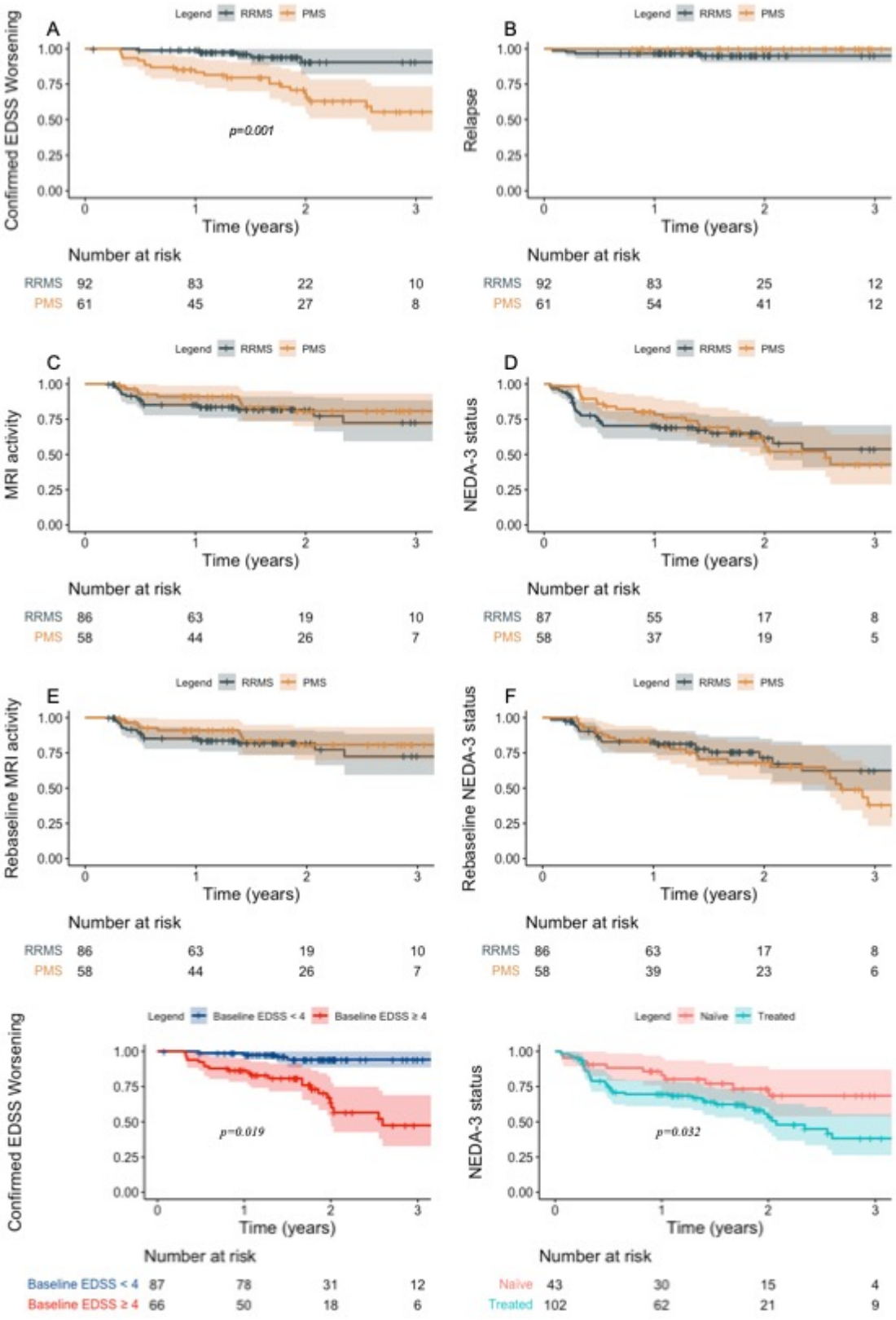
| | | | **PPMS** (n=43) | **SPMS** (n=17) | **Total PMS** (n=60) |
|---|---|---|---|---|---|
| Females, n (%) | 84 (54.9) | 60 (64.5) | 20 (46.5) | 4 (23.5) | 24 (40.0) |
| Age, mean (SD), y | 41.9 (11.4) | 36.9 (10.2) | 49.2 (8.6) | 50.6 (8.2) | 49.6 (8.4) |
| Disease duration, mean (SD), y | 10.3 (9.9) | 9.3 (9.2) | 8.4 (6.5) | 20.8 (14.7) | 11.9 (11.0) |
| EDSS, median (IQR) | 3.5 (2-5.5) | 2 (2-3.5) | 5.5 (3.5-6.5) | 6 (5-6.5) | 6 (3.5-6.5) |
| Number of relapses in previous 12 months, mean (SD) | 0.5 (0.7) | 0.8 (0.7) | - | 0.2 (0.4) | 0.1 (0.2) |
| MRI activity at ocrelizumab start, n (%) | 91 (59.5%) | 75 (81.5) | 9 (21.4) | 7 (41.2) | 16 (27.1) |
| Number of previous treatment, median (IQR) | 1 (0-2) | 1 (0-3) | 1 (0-1) | 1 (1-3) | 1 (0-2) |
| Naïve patients, n (%) | 46 (30.1) | 25 (26.9) | 18 (41.9) | 3 (17.6) | 21 (35.0) |
| Previous exposure to high efficacy DMT, n (%) | 58 (54.2) | 44 (64.7) | 7 (28.0) | 7 (50.0) | 14 (35.9) |
| Last DMT, n (%) | | | | | |
| Interferon | 12 (7.8) | 7 (7.5) | 4 (9.3) | 1 (5.9) | 5 (8.3) |
| Glatiramer acetate | 10 (6.5) | 4 (4.3) | 3 (7.0) | 3 (17.6) | 6 (10.0) |
| Fingolimod | 26 (17) | 24 (25.8) | 1 (2.3) | 1 (5.9) | 2 (3.3) |
| Dimethyl fumarate | 13 (8.5) | 6 (6.5) | 5 (11.6) | 2 (11.8) | 7 (11.7) |
| Teriflunomide | 4 (2.6) | 4 (4.3) | 0 (0) | 0 (0) | 0 (0) |
| Natalizumab | 9 (5.9) | 6 (6.5) | 0 (0) | 3 (17.6) | 3 (5.0) |
| Alemtuzumab | 11 (7.2) | 10 (10.8) | 0 (0) | 1 (5.9) | 1 (1.7) |
| Cladribine | 2 (1.3) | 2 (2.2) | 0 (0) | 0 (0) | 0 (0) |
| Other | 20 (13.1) | 5 (5.4) | 12 (27.9) | 3 (17.6) | 15 (25) |
| Time from DMT discontinuation to ocrelizumab start, median (IQR), d | 68 (30-501) | 60 (25-112) | 335 (81-1157) | 859 (132-2158) | 452 (99-1228) |
| Aggressive MS, n (%) | 17 (11.1) | 14 (15.1) | - | 3 (17.6) | 3 (5.0) |
| Advanced MS, n (%) | 66 (43.1) | 22 (23.7) | 30 (69.8) | 14 (82.4) | 44 (73.3) |
| Follow-up, median (IQR) y | 1.9 (1.3-2.7) | 1.6 (1.11-2.03) | 2.5 (2.0-3.0) | 2.0 (1.2-2.7) | 2.3 (1.8-2.99) |

*Outcomes*

Figure 1 (panel A–F) reports the results of the primary outcome. At 2-year FU, 90.5% of patients with RRMS, 64.7% of patients with PPMS, and 68.8% of patients with SPMS were free of disability worsening. Two out of five patients with RRMS who experienced disability worsening had highly active MS and 5/5 experienced progression independent of relapse activity. A total of 95.1% patients with RRMS were free of relapses at 2-year FU. Four patients had a single relapse at + 27, + 72, + 103, and + 520 days, respectively. In the RRMS cohort, pre-treatment annualized relapse rate (ARR) was

0.78 (0.70), 1-year FU ARR was 0.04 (0.18), and 2-year FU ARR was 0.04 (0.21). At 2-year FU, 67.1% RRMS patients, 81.3% PPMS patients, and 72.7% SPMS patients were free of MRI evidence of disease activity. After a 100-day re-baseline of MRI activity, percentages of patients without MRI activity increased to 82.1%, 83.8%, and 72.7% for RRMS, PPMS, and SPMS patients respectively. After a 180-day re-baseline, percentages increased to 92.1%, 88.4%, and 83.1%. Male sex, RRMS phenotype, previous exposure to DMT, and baseline active MRI were associated with an increased risk of MRI activity during ocrelizumab therapy. At multivariate analysis, patients previously treated with a DMT had an increased risk of MRI activity (HR (95%CI) = 2.53 (1.05–6.10), p = 0.039). Among 21 patients who experienced MRI inflammatory activity within 180 days, only 1 patient had further MRI activity at + 520 days. At 2-year FU, NEDA-3 percentages were 62.1%, 54.6%, and 55.1% for RRMS, PPMS, and SPMS respectively. After a 100-day re-baseline of MRI activity, NEDA-3 percentages increased to 71.9%, 71.4%, and 57.9% respectively. NEDA-3 rates after a 180-day re-baseline were 77.1%, 71.9%, and 67.3% for RRMS, PPMS, and SPMS respectively. Naïve patients had a higher probability of achieving NEDA-3 status (HR 0.48 (0.25–0.94), p = 0.032) (Fig. 1, panel H).

Figure 1. NEDA-3 status and individual components during ocrelizumab therapy in relapsing-remitting and progressive MS



*Ocrelizumab in patients with Highly Active MS*

Patients with highly active MS had a higher risk of persistent MRI inflammatory activity (MRI-free survival 58.3% vs 75.5%, HR = 0.35 (0.15–0.82), p = 0.015) and tended to have lower NEDA-3 rates (NEDA-3 percentage of 38.9% vs 60.1, HR = 0.48 (0.22–1.02), p = 0.056) compared to patients without the same baseline characteristics.

*Predictors of Early Inflammatory Activity*

Out of 145 patients, 30 (20.7%) had early inflammatory activity in the first year of treatment. The RRMS phenotype (OR 3.19 (1.21–8.39), p = 0.019) and MRI activity at baseline (OR 0.21 (0.07–0.59), p = 0.003) were associated with an increased risk of early inflammatory activity. Leukocyte, total lymphocyte, CD4+, CD8+, and CD19+ cell counts during the first year of ocrelizumab therapy are reported in Table 2.

Table 2. Lymphocyte subsets during the first year of treatment.

| | **Total Cohort** | **Treated** | **Treatment-Naive** | **Treated vs Naive p value^** | **With early inflammatory activity** | **Without early inflammatory activity** | **With vs without early inflammatory activity p value°** |
|---|---|---|---|---|---|---|---|
| **Baseline** | | | | | | | |
| Available, n | 73 | 55 | 18 | | 17 | 56 | |
| Leucocytes, N/mm$^3$ | 6273 (2262) | 6064 (2208) | 6913 (2368) | 0.254 | 6501 (3232) | 6205 (1908) | 0.522 |
| Lymphocyte, N/mm$^3$ | 1793 (771) | 1680 (801) | 2139 (557) | 0.050 | 2030 (1188) | 1721 (587) | 0.088 |
| CD3+, N/mm$^3$ | 1259 (602) | 1158 (624) | 1568 (404) | 0.022* | 1452 (879) | 1201 (484) | 0.085 |
| CD4+, N/mm$^3$ | 807 (446) | 742 (463) | 1006 (323) | 0.047* | 922 (641) | 772 (367) | 0.172 |
| CD8+, N/mm$^3$ | 439 (214) | 411 (224) | 526 (153) | 0.088 | 515 (263) | 416 (193) | 0.064 |
| CD19+, N/mm$^3$ | 291 (257) | 289 (244) | 299 (303) | 0.960 | 334 (366) | 279 (217) | 0.407 |
| **6 months** | | | | | | | |
| Available, n | 73 | 55 | 18 | | 17 | 56 | |
| Leucocytes, N/mm$^3$ | 5712 (1666) | 5681 (1737) | 5806 (1469) | 0.870 | 5991 (1386) | 5627 (1745) | 0.209 |
| Lymphocyte, N/mm$^3$ | 1388 (547) | 1306 (554) | 1639 (451) | 0.036* | 1564 (666) | 1335 (501) | 0.049* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| CD3+, N/mm$^3$ | 1122 (505) | 1042 (508) | 1367 (419) | 0.027* | 1327 (617) | 1060 (454) | 0.014* |
| CD4+, N/mm$^3$ | 714 (367) | 652 (366) | 903 (309) | 0.016* | 824 (446) | 680 (338) | 0.046* |
| CD8+, N/mm$^3$ | 369 (192) | 352 (196) | 421 (176) | 0.305 | 484 (214) | 334 (173) | 0.001** |
| CD19+, N/mm$^3$ | 19 (32) | 23 (36) | 7 (11) | 0.084 | 29 (46) | 17 (27) | 0.495 |
| **12 months** | | | | | | | |
| Available, n | 61 | 45 | 16 | | 11 | 50 | |
| Leucocytes, N/mm$^3$ | 6306 (2704) | 6296 (3055) | 6334 (1314) | 0.912 | 5432 (1499) | 6532 (2926) | 0.482 |
| Lymphocyte, N/mm$^3$ | 1488 (655) | 1359 (669) | 1868 (476) | 0.022* | 1513 (561) | 1528 (661) | 0.584 |
| CD3+, N/mm$^3$ | 1204 (524) | 1091 (496) | 1525 (476) | 0.012* | 1233 (493) | 1211 (533) | 0.469 |
| CD4+, N/mm$^3$ | 780 (356) | 695 (328) | 1020 (330) | 0.003* | 760 (320) | 794 (365) | 0.759 |
| CD8+, N/mm$^3$ | 397 (224) | 370 (228) | 471 (199) | 0.251 | 456 (314) | 387 (201) | 0.199 |
| CD19+, N/mm$^3$ | 25 (70) | 32 (81) | 5 (7) | 0.267 | 11 (14) | 28 (78) | 0.314 |

While no significant differences in cell count were present at baseline, at 6-month FU, patients with early inflammatory activity had higher total lymphocytes, CD3+, CD4+, and CD8+ cell counts compared with stable patients. Only difference in CD8+ cell count survived Bonferroni correction for multiple comparisons. Using linear mixed model, CD8+ cell decrease at 6-month FU was less pronounced in patients with early inflammatory activity ($p = 0.022$), while no significant differences were noted in the dynamics of CD4 + and CD19 + counts. When including CD4+, CD8+, and CD19 + cell counts to MS phenotype and MRI baseline activity in a logistic regression model predicting early inflammatory activity, CD8+ count was the only independent variable associated with outcome (OR 1.005 (1.001–1.009), $p = 0.019$).

*Discussion*

We herein provide single-center effectiveness and safety data about ocrelizumab treatment in relapsing–remitting and progressive MS patients, prospectively followed in a real-world setting for a median follow-up of almost 2 years. Our cohort consisted of a heterogeneous group of patients with a large variety in terms of age, disease activity, phenotype, treatment history, and comorbidities, and included a relatively high number of patients with recent high disease activity. In the RRMS population we observed that, at 2-year FU, 95.1% patients were free of relapses, 90.5%

patients free of disability worsening, and 67.1% patients free of MRI activity, with an overall percentage of patients reaching NEDA-3 of 62.1%. The ARR decreased from 0.78 to 0.04 (at 1- and 2-year FU), which is in line with the relapse rate at 2-year FU reported in the OPERA I and II studies (0.16)(Hauser et al., 2020). Despite such encouraging results, we observed some evidence of persistent MRI activity in the first year of treatment, both in RRMS and PMS patients (MRI activity-free survival at 2 years of 67.1%, 81.3%, and 72.7% for RRMS, PPMS, and SPMS patients, respectively). The post hoc analysis pooling results of phase II and III trials suggested that ocrelizumab efficacy in terms of MRI outcome is evident as early as 4 weeks, and nearly complete by week 8(Barkhof et al., 2019). We performed a re-baseline of MRI activity at 100 and 180 days after ocrelizumab start and observed that percentages of MRI activity free patients increased to 73–82% and to 83–92%, respectively. Accordingly, NEDA-3 percentages after 180 days of treatment increased to 77.1%, 71.9%, and 67.3% for RRMS, PPMS, and SPMS respectively. These results are in line with the post hoc analysis of the OPERA trial, in which the proportion of patients reaching NEDA-3 status was 72.2% after a 24-week re-baseline. In RRMS patients, the presence of inflammatory activity at baseline MRI and a previous DMT exposure were associated with an increased risk of MRI activity during ocrelizumab treatment, with previous DMT exposure being the only independent variable associated with worse outcome at the multivariate analysis. These results are in line with those from an independent cohort of patients treated with ocrelizumab, in which lower baseline EDSS and higher previous relapse rate were associated with an increased risk of early inflammatory activity during ocrelizumab treatment (Signoriello et al., 2022).

We also observed significantly lower rates of MRI activity free survival (58.3%) in the subgroup of patients with highly active MS, compared to the total cohort of patients. Since a rapid effect in controlling MRI activity is critical to minimize brain damage and prevent accumulation of disability in aggressive MS, these results suggest that in patients in whom immediate and complete disease control is warranted, DMTs with more rapid immune-ablative action should be considered.

It is generally believed that the impairment of the antigen presenting capacity of B cells is one of the major mechanisms underlying the therapeutic efficacy of ocrelizumab. However, a subset of T cells, mainly CD8+, also expresses CD20 and represents a highly activated cell population CD20+ T cells have been found to be increased in MS patients, representing almost 20% of all CD20+ expressing cells (Gingele et al., 2018). It has been recently showed that ocrelizumab reduces CD20+ T cells after 6 months of treatment, in line with similar findings found during treatment with rituximab (Fernández-Velasco et al., 2021).

We showed that patients with persistent early inflammatory activity during the first year of treatment had higher levels of CD8 + cells at 6-month FU as compared with stable patients. Although our evidence is limited, we cautiously speculate that the decrease in CD8 + cells is driven, at least partially, by the reduction of CD20 + T cells induced by ocrelizumab. Indeed, a transient reduction in CD8 + cells has been reported in phase III trials and in real-life patients (Capasso et al., 2021), but to date no evidence exists on the possible therapeutic effect of this cellular subset reduction. Since these findings are based on immunophenotyping data collected in clinical practice, we could not validate them with double staining analyses and thus provide a mechanistic explanation for our observations. Larger studies exploring the effect of ocrelizumab treatment on CD20 + CD8 + cells and its possible association with clinical outcomes are needed to confirm our findings.

*Limitations*

Our study has several limitations, including the lack of spine MRI, patients' BMI, and a relatively short follow-up. In addition, flow cytometry immune subset characterization was available only for a subgroup of patients. Finally, pre-treatment brain MRI were acquired within 3 months of ocrelizumab start and not just before treatment commencement; thus, we cannot rule out whether part of the persistent MRI activity we observed during the first months of treatment occurred before treatment start.

## 2.5 Impact of highly active immunotherapy on acute and chronic neuroinflammation in aggressive multiple sclerosis

*Background and aims*

Aggressive multiple sclerosis, which affect 4-14% of MS patients, is characterized by severe relapses, accelerated accrual of disability and poor response to treatment (Iacobaeus et al., 2020). To date, no evidence-based criteria exist to guide the choice on the best treatment approach in these patients. As a result, the recently updated/published guidelines from the American Academy of Neurology (Rae-Grant et al., 2018) and the European Academy of Neurology/European Committee on Treatment and Research in MS (Montalban et al., 2018) do not address the management of patients with aggressive MS. Several disease modifying therapies (DMTs) are used in clinical practice in patients with highly active MS, such as alemtuzumab, natalizumab, ocrelizumab and cladribine. Moreover, in the last 10 years, intense immunosuppression followed by autologous hematopoietic stem cell transplantation (AHSCT) has been increasingly explored as a treatment strategy for aggressive MS. The rationale of AHSCT in MS is to eliminate self-reacting cell clones and to induce long-term self-tolerance through a profound renewal of the immune system (Mancardi et al., 2018). To date, only two controlled studies have compared AHSCT with highly active DMTs in patients with aggressive MS (Burt et al., 2019b; Mancardi et al., 2015), suggesting that AHSCT allows more complete disease control compared to standard DMTs.

However, these studies lack robust MRI analyses aimed at investigating the effect of treatments on advanced metrics of acute and chronic neuroinflammation, limiting our comprehension of the real extent of the immunosuppressive effects of the different treatments. In particular, the impact of available highly active DMTs and AHSCT on chronic smoldering inflammation, which is now recognized as a main driver of disability progression in MS, is still unknown. Recent advanced MRI techniques hold promise to visualize in vivo compartmentalized chronic neuroinflammation. Susceptibility MRI allows identification of paramagnetic rim lesions (PRLs), reflecting MS lesions with a peripheral rim of activated macrophages/microglial cells loaded with iron. Susceptibility MRI is thus a potential candidate to monitor treatment response, as recently reported during treatment with dimethyl-fumarate (Eisele et al., 2022; Zinger et al., 2022). Of interest, to date no study has specifically addressed PRLs evolution after AHSCT. Two pathological studies of MS lesions in patients who underwent AHSCT demonstrated an almost complete suppression of brain lymphocytes and

macrophages/microglial cells (Metz et al., 2007; Wundes et al., 2017) in the short and long-term follow-up.

Finally, in addition to the assessment of new/active and smoldering lesions, MRI can be used to monitor the impact of immunotherapies in MS by tracking quantitatively changes in the normal appearing structures of the brain. For example, diffusion imaging can be used to measure changes in the integrity of white matter, providing information on the impact of immunotherapies on diffuse myelin and axonal damage.

We organized this prospective observational study including consecutive patients affected by aggressive MS and treated with highly active DMTs, including AHSCT. Patients were scanned with a standardized quantitative MRI protocol at disease breakthrough (before treatment start) and at 6, 12 and 24 months.

The main aim of this study was to assess and compare the impact of different highly active immunotherapies, in reducing acute and chronic neuroinflammation. Main objectives were to analyze the occurrence and the evolution of paramagnetic rim lesions and the degree of microstructural damage in the normal-appearing white matter.

*Methods*

Consecutive MS patients affected by aggressive MS and treated with highly active DMTs from the MS Center of the University of Genoa were included in this ongoing study.

All patients had recent (<30 days) MS relapse with EDSS increase of at least 1 point while on standard DMT for at least 6 months and the occurrence of at least one gadolinium enhancing lesion at brain/spine MRI. We also included treatment naïve patients in presence of negative prognostic factors indicating the necessity to start a highly active DMT (spinal cord lesions, multiple early relapses and/or accelerated disability progression).

Subjects have been scanned with a 3T Siemens Prisma Scanner equipped with a 64 channels head coil at disease breakthrough and at 6, 12 and 24 months. MRI protocol included:

-Fluid attenuation inversion recovery (repetition time -TR-/inversion time-TI/echo time -TE-: 5000/1800/393 ms; resolution 0.4×0.4×1 mm)

-3D sagittal T1 MPRAGE (TR/TE: 2300 ms/919 ms/2.96 ms; resolution 1 × 1 × 1 mm 3)

-Sagittal segmented echo-planar imaging (EPI) providing T2* magnitude and phase contrasts (TR/TE: 64/35 ms; resolution 0.65×0.65×0.65 mm3)

-Twice-refocused spin echo echo-planar imaging sequence for multi-shell diffusion-weighted images (TR/TE:4500/75 ms; 107 diffusion directions distributed in 5 shells with b-value up to 3000s/mm2 plus 7 non weighted images acquired with both anterior-posterior and posterior-anterior phase encoding directions; resolution 1.8×1.8×1.8 mm3)

-T1 space post gadolinium injection (TR/TE: 700/12 ms; resolution 1×1×1 mm3)

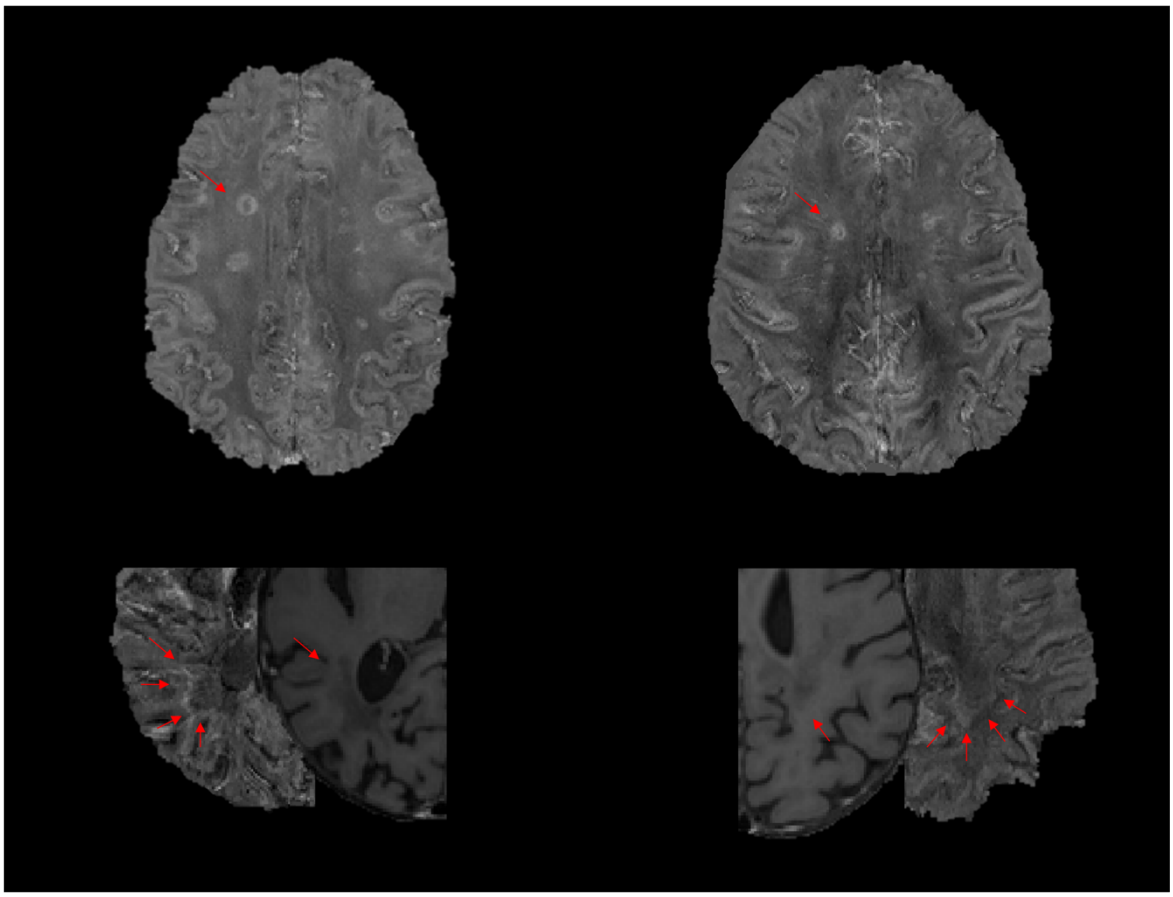
*MRI preprocessing*

The presence of gadolinium enhancing/new MS lesions was inspected by one experienced neuroradiologists. FLAIR lesions were manually segmented using Jim (Xinapse System).

After MS lesion filling of the high-resolution T1, total brain, grey matter and white matter volumes were obtained with SPM12. Brain volume changes were assessed using SIENA, part of FSL. Magnetic susceptibility maps were obtained from 3D-EPI using a custom Matlab code (The MathWorks Inc.,Natick,USA) with STIsuite routines for phase unwrapping, background phase removal and dipole deconvolution. A neurologist with experience in neuroimaging (> 5 years) identified paramagnetic rim lesions in all scans (after alignment of all MRI scans to a half-way-space using ANTs), excluding gadolinium-enhancing lesions. Some examples of PRLs in this population are shown in Figure 1.

dMRIs were pre-processed using a combination of FSL and MRtrix3 following these steps: denoising, movement artifacts and susceptibility induced distortions removal, B1-bias correction. We subsequently extracted the intra-axonal signal fraction (SMT-intra), and the mean and transversal diffusivities (SMT-extramd and SMT-extratrans) of multicompartment spherical mean technique (SMT) using Kaden's official toolbox.

Figure 1. Examples of paramagnetic rim lesions in patients with aggressive multiple sclerosis.



*Study population*

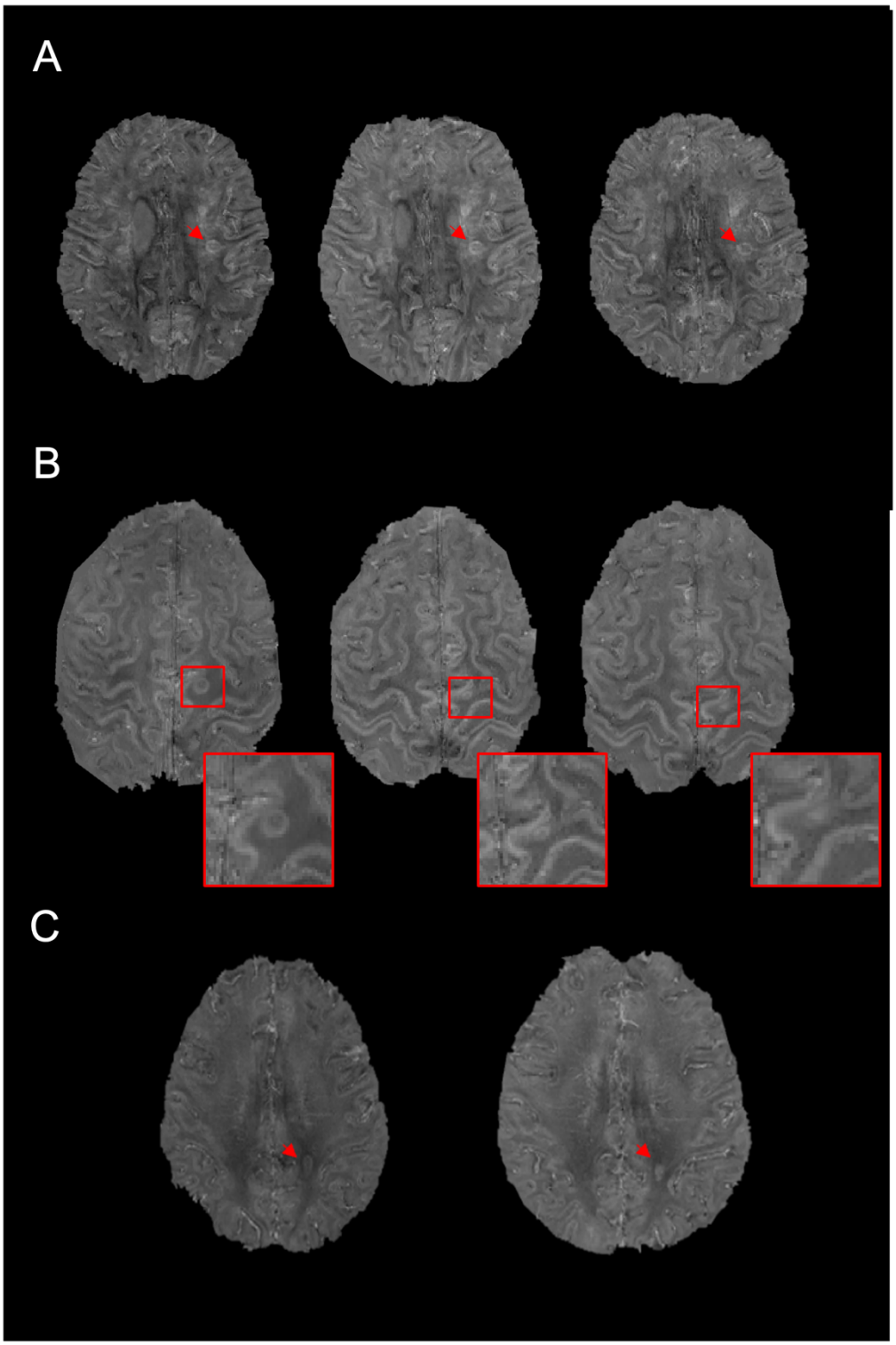
Table 1 reports the baseline characteristics of the study population. We included 8 people with aggressive MS who underwent AHSCT, and 11 patients treated with highly active DMTs (ocrelizumab, natalizumab and cladribine). All transplanted patients experienced a breakthrough disease activity while on DMT, while 5 patients in the "other DMTs" group were treated naïve. Mean number of gadolinium enhancing lesions was 8 in the AHSCT group and 3 in the "other DMTs" group. For 4/8 transplanted patients, longitudinal assessment of PRLs was not possible (n=2 bad baseline image quality, n=2 T2* magnitude and phase contrasts not acquired in the MRI protocol).

Table 1. Baseline demographic, clinical and MRI characteristics

| | AHSCT (n=8) | Highly active disease modifying therapies (n=11) | p-value |
|---|---|---|---|
| Age, y, mean (SD) | 37 (7) | 38 (11) | ns |
| Female, n (%) | 7 (87) | 8 (73) | ns |
| EDSS, mean (SD) | 5.1 (1.0) | 3.2 (1.5) | 0.02 |
| Disease duration, y, mean (SD) | 12.8 (8.0) | 8.1 (7.5) | ns |
| Active Progressive MS, number (%) | 2 (25) | 1 (9) | ns |
| Disease breakthrough on DMT, n (%) | 8 (100) | 6 (55) | 0.02 |
| Therapy, name (number) | - | • Ocrelizumab (5)<br>• Natalizumab (3)<br>• Cladribine (3) | - |
| FLAIR lesion volume, mL, mean (SD) | 30.0 (25.0) | 17.4 (13.1) | ns |
| Gadolinium enhancing lesion, mean (SD) | 8 (11) | 3 (2) | ns |
| Normalized brain volume, mL, mean (SD) | 1293 (121) | 1368 (153) | ns |

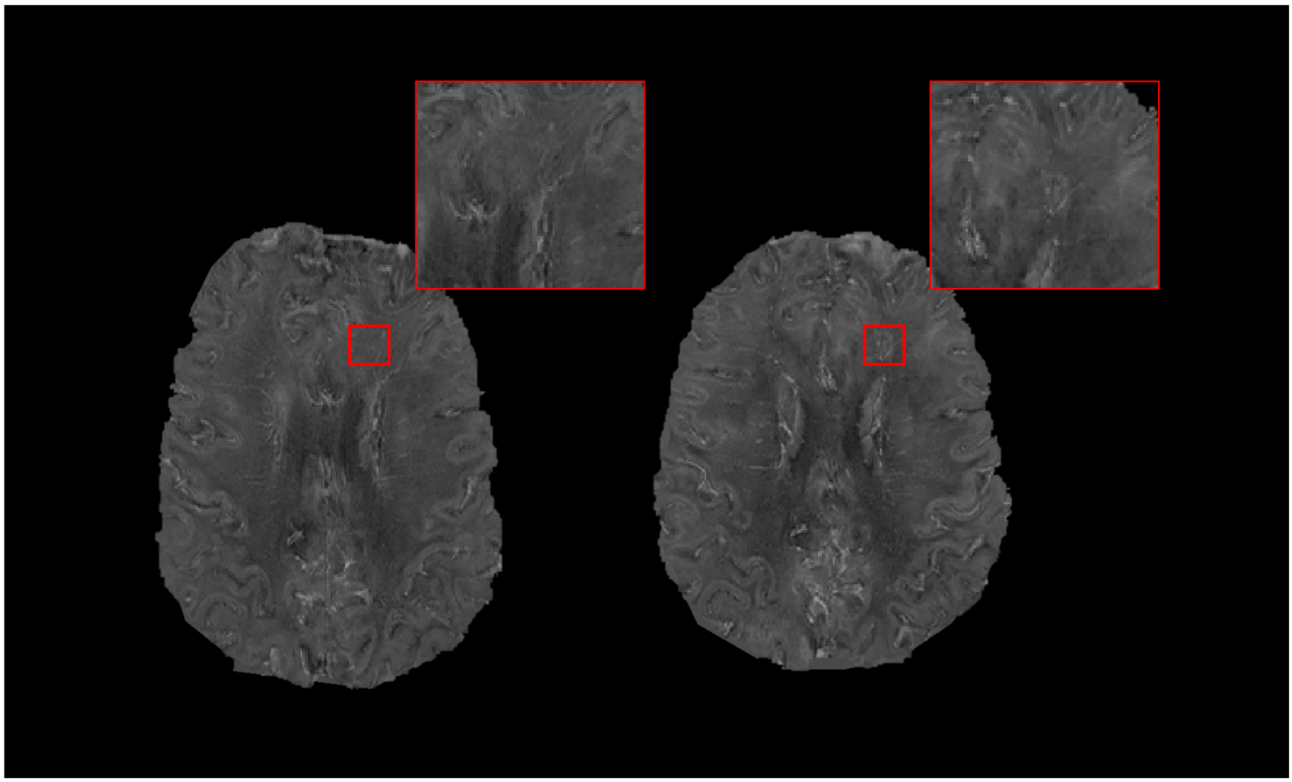
At baseline, 12 patients (80%) exhibited at least one PRL, with a median number of PRL per patient of 2 (IQR=1-3). In each subject, PRLs represented in mean the 5% (IQR=1.5-10%) of total MS lesions. Over 2 years, none of AHSCT patients had new/gadolinium-enhancing MS lesions, compared to 4 patients (36%) in the “other DMTs” group. Most of the PRLs persisted unchanged over time in both treatment groups. In 3 patients (2 AHSCT treated patients, 1 treated with cyclophosphamide followed by ocrelizumab) a reduction in rim intensity or a change in the QSM hyperintensity pattern change was observed. Figure 2A shows a PRL remaining stable over 2 years in a transplanted patient, figure 2B and 2B show two PRLs exhibiting a reduction in rim intensity/change in hyperintensity pattern.

Figure 2. Dynamics of paramagnetic rim lesions under highly active treatments. Figure 2A shows a PRL remaining stable over 2 years in a transplanted patient, figure 2B and 2B show two PRLs exhibiting a reduction in rim intensity/change in hyperintensity pattern in transplanted patients and a patient treated with ocrelizumab, respectively.



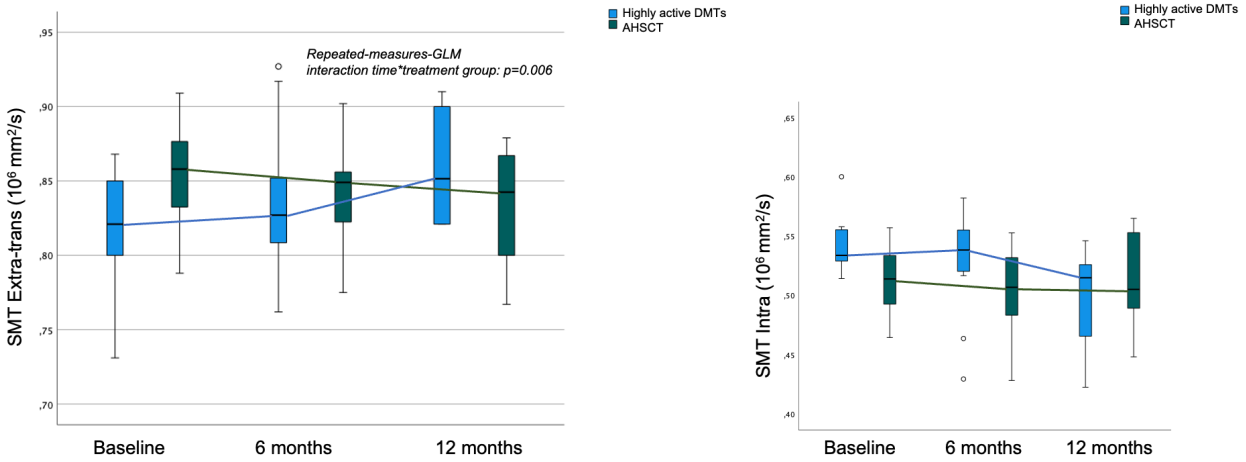
Over 2 years, none (0/4) of AHSCT patients developed a new PRL, while they appeared in 2/11 patients in the highly active DMT group (Figure 3).

Figure 3. A new lesion with paramagnetic rim occurring during treatment with natalizumab.



Finally, we analyzed the dynamics of the diffusion-derived metrics in the normal-appearing white matter in the two treatment groups. Figure 4 reports the boxplot of intra-axonal signal fraction (SMT-intra), and the transversal diffusivities (SMT-extra trans) derived from multicompartment spherical mean technique. NAWM of patients treated with other DMTs showed increased extra-trans values over 1 year, while slightly decreased/stabilized in the transplanted patients (repeated measures GLM, interaction time*treatment group p=0.006). No significant differences were noted in the SMT-intra values.

Figure 4. Intra and extra-axonal diffusion fractions after treatment start.




*Conclusions*

Results from this study confirm that in people with aggressive MS, highly active DMTs are associated with a significant reduction of acute CNS inflammation, with AHSCT being associated with the strongest effect on both focal and diffuse neuroinflammation. No active MS lesions appeared after AHSCT, which was also associated with the most profound impact on diffuse inflammation in the normal appearing white matter. Diffusion derived metrics of axonal and myelin integrity ameliorated in AHSCT group starting from the first months after the procedure. Since a rapid effect in controlling MRI activity is critical to minimize brain damage and to prevent accumulation of disability in aggressive MS, these results support the idea that in patients in whom immediate and complete disease control is warranted, rapid immune-ablative action achieved by AHSCT could be a valuable option.

Interestingly, we confirm that PRLs are frequent in patients with an aggressive disease course, being detectable in 80% of our cohort. Such rates are higher the ~50% reported in other studies analyzing mixed cohort of patients (Absinta et al., 2016, 2013; Bagnato et al., 2011; Dal-Bianco et al., 2021, 2017; Hammond et al., 2008; Maggi et al., 2020). While it is known that PRLs do occur from the beginning until the late phases of multiple sclerosis, some authors found that PRLs are more frequent in RRMS (Dal-Bianco et al., 2021), while others found higher rates of PRLs in progressive MS (Absinta et al., 2019). According to our results, it is likely that these discrepancies are due to the proportion of patients with an aggressive disease course included in the different studies. Moreover,

this finding highlights the potential role of PRLs, which are known to be associated with an increased risk of disability worsening (Absinta et al., 2019; Maggi et al., 2021), in selecting candidate patients for AHSCT.

Our results show that PRLs tend to persist over time, despite the use of highly active immunosuppression, including AHSCT. These results are in line with several studies, showing that PRLs tend to remain stable despite active treatment. In a long-term follow-up study of PRLs (Dal-Bianco et al., 2021), most patients were treated with a first-line or a second-line DMT (75%) and no effect of treatment was noted in PRLs evolution/disappearance. Similarly, TSPO-PET studies evaluating the impact of highly active therapies (Sucksdorff et al., 2019, 2017), including rituximab (Lehto et al., 2022), on rim lesions, showed a slight reduction of the PET signal within the rim, rather than a complete suppression of PRLs. Similarly, we found that some PRLs exhibit changes in the intensity and the distribution of QSM signal over time, which could be related, at least in part, to highly active CNS penetrant drugs treatment used in these patients (BEAM+ATG transplant in 2 patients and cyclophosphamide + ocrelizumab in one patient). Quantitative analysis of QSM signal, rather than simple qualitative assessment, might be more sensible to detect changes in chronic infiltrates within PRLs and should be performed in future studies. It should be finally remembered here that iron accumulation after inflammatory demyelination may contribute to lesion repair rather than inflammatory demyelination per se, as a consequence of an increased perivascular iron deposition and increase uptake by oligodendrocytes during remyelination.

It is noteworthy that no PRLs evolved during follow-up in patients treated with AHSCT or ocrelizumab, while one lesion became PRL under natalizumab treatment. If confirmed, these results could in part explain the long-term effects of these treatment on disability evolution. However, bigger sample sizes and longer follow-ups are needed to confirm these preliminary findings.

## 2.6 Deep multiple sclerosis lesion phenotyping using multimodal quantitative MRI

Abstract

Quantitative MRI has the potential to disentangle the heterogeneity of multiple sclerosis (MS) lesions in vivo, which can be decisive for treatment and monitoring. In this study, we distinguished different types of MS lesions based on quantitative susceptibility mapping (QSM) and characterized them using T1 relaxometry, diffusion imaging and myelin mapping. We identified four types of lesions (hypo-isointense, homogeneous hyperintense, inhomogeneous hyperintense and paramagnetic rim), which were characterized by increasing degrees of axonal and myelin disruption. Paramagnetic rim lesions were closer to the periventricular CSF, corroborating the presence of a noxious activity of CSF in MS pathology.

*Background and aims*

Neuropathological studies have shown that multiple sclerosis (MS) lesions are heterogeneous in terms of axonal and myelin damage as well as immune cells infiltrates (Kuhlmann et al., 2017). In QSM, MS lesions may appear hyper-, iso-, or hypointense, compared to the surrounding tissue. Particularly, QSM hypo- and isointense lesions have been recently described as a potential biomarker for remyelinating lesions (Rahmanzadeh et al., 2022). Conversely, QSM hyperintense lesions seem to correspond to more destructive lesions. Of note, the hyperintense QSM signal may be confined to the borders of the lesion (paramagnetic rim lesions) or distributed within the lesion itself. Lesion localization has been proposed as one of the possible responsible for lesion heterogeneity, as lesions closer to the ventricular CSF seem to be more destructive and less likely to undergo remyelination, while remyelinated lesions are more likely in the deep white matter (Tonietto et al., 2022).

For a more comprehensive classification and a better understanding of lesion heterogeneity in MS, in this study we aimed to:

i. Identify different MS lesion types using QSM and to characterize them using relaxometry, myelin mapping, and diffusion MRI.
ii. Characterize the spatial distribution of different lesion types with respect to the ventricular CSF.

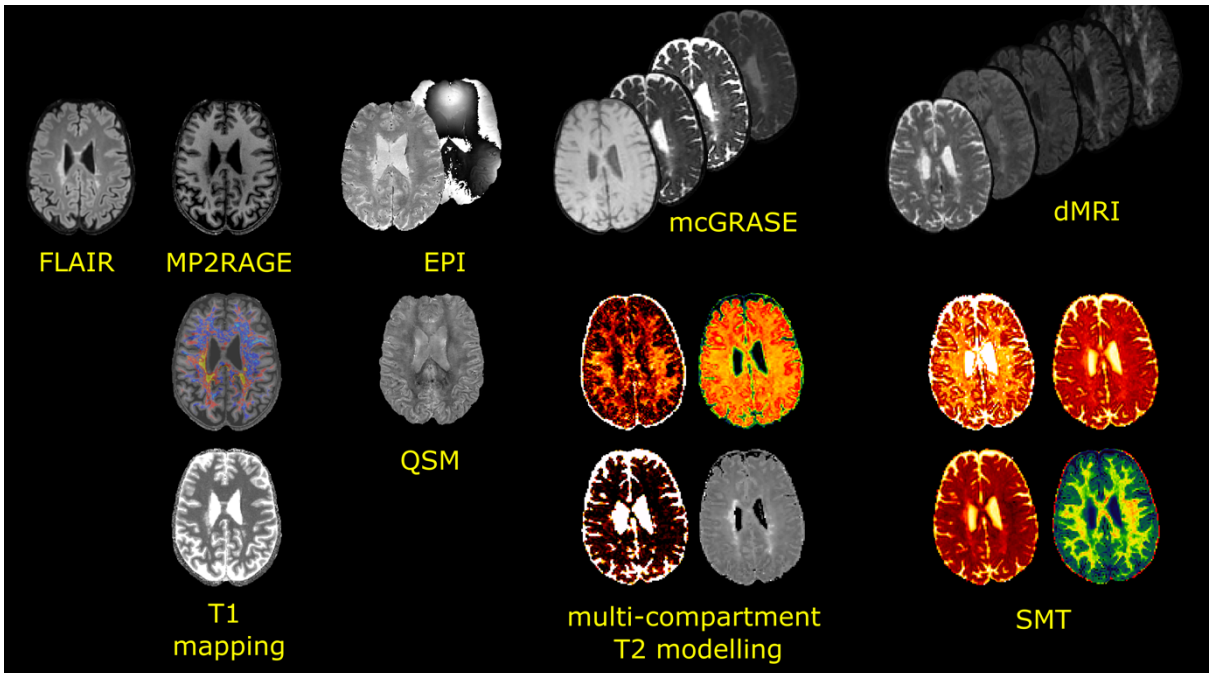
*Methods*

Fifty-two people with MS (34 females, age=44, interquartile range -IQR- 32-52, 40 relapsing-remitting -RR- and 12 secondary progressive -SPMS-) and 24 healthy controls (-HC-,16 females, age=38, IQR 27-52) were scanned at 3T (MAGNETOM Prisma, Siemens Healthcare) using the following sequences:

- 3D-FLAIR (TR/TE/TI=5000/393/1800ms, resolution=0.4x0.4x1mm3, TA=6'37"); 4.6x accelerated compressed sensing MP2RAGE research application (TR/TE/TI1/TI2=5000/2.9/700/2500ms, resolution=1mm isotropic,TA=3'40")
- segmented 3D-EPI for QSM9 (TR/TE=64/35ms, resolution=0.65mm isotropic)
- mcGRASE10 research application (TR=1000ms,32 TE in [10.36, 331.52] ms, resolution=1.8mm isotropic, CAIPIRINHA11 3x2, TA=9'39")
- multi-shell dMRI with 107 directions and b-value up to 3000s/mm2 (TR/TE=4600/75ms, resolution=1.8mm isotropic, TA=8'58") and 12 measurements of b-value 0s/mm2 with reversed phase encoding.

Figure1 illustrates the native and derived images.

Figure 1. Selected axial slices of the protocol MRI sequences and the relative quantitative maps




FLAIR lesions were automatically segmented using SinLab (SienaImaging,Italy), while WM masks were obtained using FreeSurfer and removal of subcortical nuclei segmented through FIRST. From the qMRI, we derived the following: averaged T1 from MP2RAGE; magnetic susceptibility maps from 3D-EPI using a custom Matlab code (The MathWorks Inc.,Natick,USA) with STIsuite routines for phase unwrapping, background phase removal and dipole deconvolution; average T2 of intra- and extra-axonal space, myelin, intra- and extra-axonal and free water fractions (MWF, IEWF and FWF) from mcGRASE. dMRIs were pre-processed using a combination of FSL and MRtrix3 following these steps: denoising, movement artifacts and susceptibility induced distortions removal, B1-bias correction. We subsequently extracted the intra-axonal signal fraction (SMT-intra), and the mean and transversal diffusivities (SMT-extramd and SMT-extratrans) of multicompartment spherical mean technique (SMT) using Kaden's official toolbox.

*Lesions classification*

We identified MS lesions based on T2-FLAIR hyperintensities and then, after affine co-registration between FLAIR and QSM, we used the corresponding QSM signal to classify lesions as hypo-isointense versus hyperintense. The latters were further classified according to the spatial pattern of QSM hyperintensity. We thus obtained 4 classes of lesions:

1.Hypo-isointense lesions

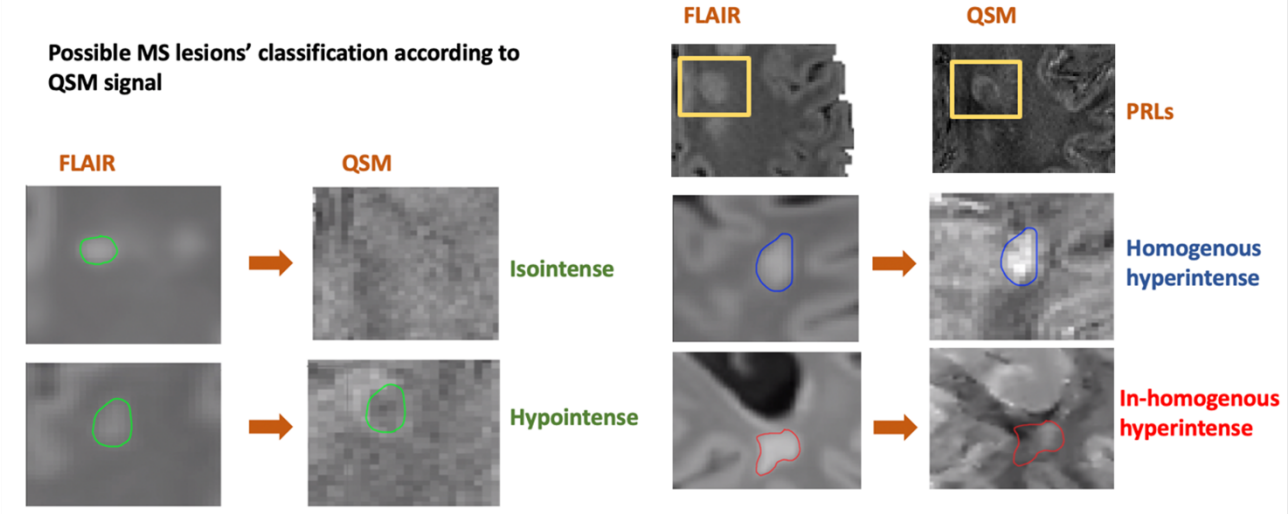
2.Homogeneous hyperintense lesions, characterized by a homogeneous distribution of hyperintensity along the whole lesion

3.Inhomogeneous hyperintense lesions, characterized by the presence of a scattered hyperintensity without a homogeneous pattern

4. Paramagnetic rim lesions (PRLs), defined by the presence of a hyperintense rim with a relative hypointense center

Figure 2 shows examples of the 4 classes of lesions.

Figure 2. Lesions classification based on QSM signal intensity and pattern




After non-linear registration of each map and lesion mask onto the MNI152 space, average microstructural metrics within the different maps were extracted inside each lesion. For QSM values, we created an atlas using the HCs maps and we computed values inside each region as:

$$\mathrm{QSM}_{\mathrm{lesion}} = \mathrm{mean}_{i \in \mathrm{lesion}}(\mathrm{QSM}_{\mathrm{patient}}(\mathrm{voxel}_i) - \mathrm{QSM}_{\mathrm{HCs}}(\mathrm{voxel}_i))$$

Distance maps from ventricular CSF towards the cortex were calculated as the 3D-Euclidean distance to the nearest nonzero voxel using the "distancemap" command (FSL).

Statistical tests were carried out using SPSS (IBM, vers 28.01), using generalized linear models controlling for patient ID, age, sex, MS phenotype and lesion volume as confounding factors.

*Results*

Out of 844 lesions, 44.4% were classified as hypo-isointense lesions (median number per subject=5, IQR=1-10), 23.0% as homogeneous hyperintense lesions (median=3, IQR=1-5), 24.9% as inhomogeneous lesions (median=4, IQR=1-6) and 7.7% as PRLs (median=1, IQR=0-2). As expected, significant differences in QSM values were noted between hypo-isointense lesions versus hyperintense lesions, while there were no differences between homogeneous/inhomogeneous/PRLs (Figure 3).

Figure 3. Error plots (95% confidence interval) of QSM values among the different lesion types

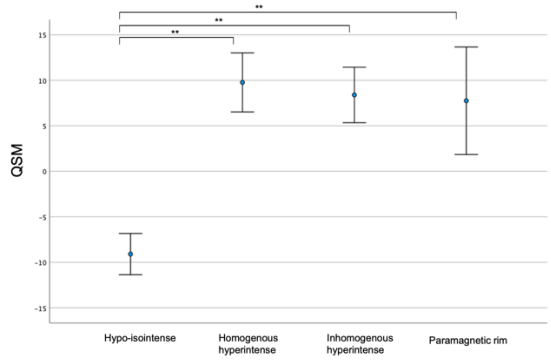
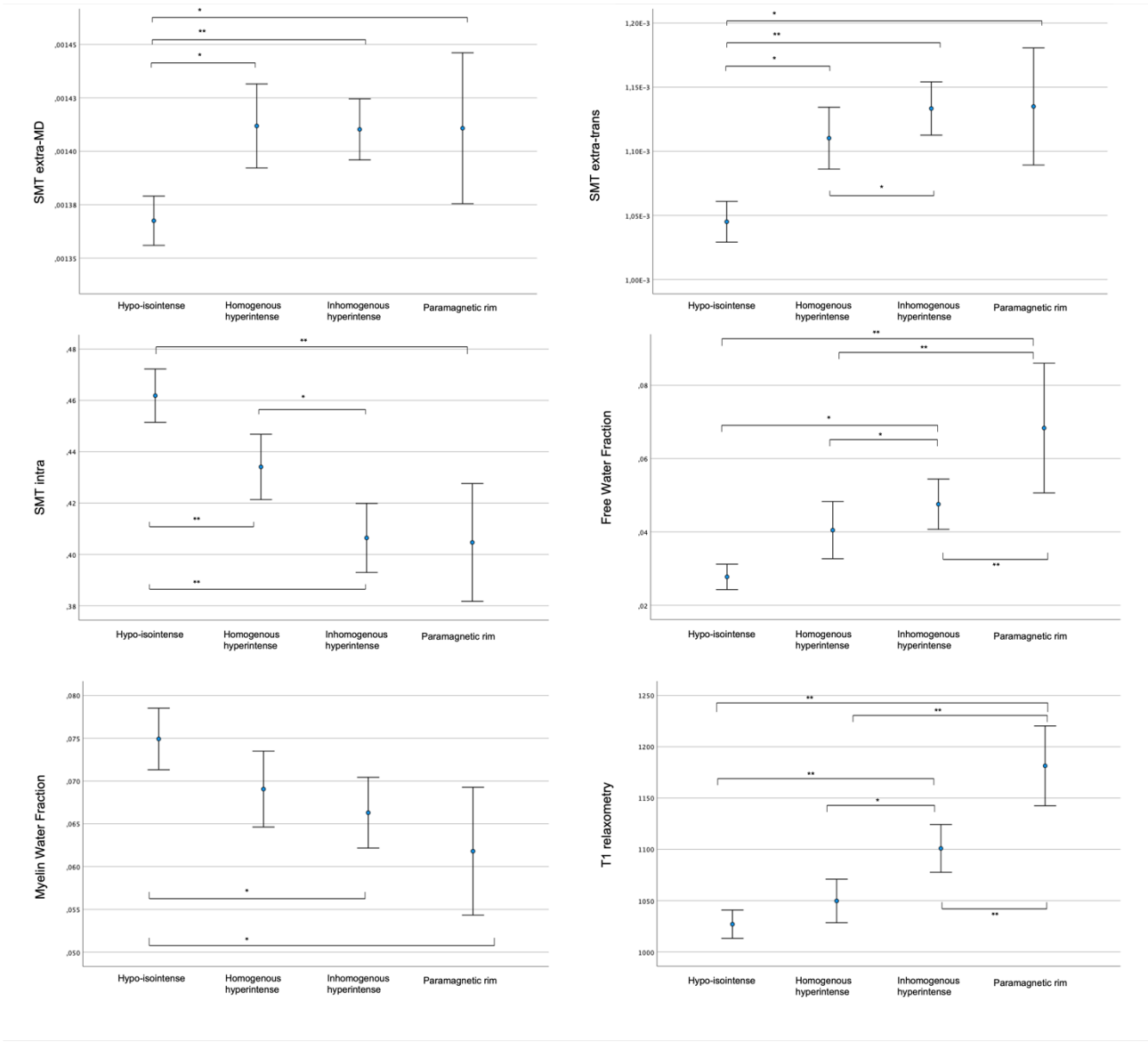



Figure 4 shows error plots of each microstructural metric among the different types of QSM lesions. A worsening gradient was noted in all SMT-derived metrics between lesion types (hypo-isointense lesions → homogeneous hyperintense lesions → inhomogeneous hyperintense lesions → PRLs). PRLs showed the highest FWF and the longer T1 values than all other lesion groups.

Figure 4. Error plots (95% confidence interval) showing diffusion MRI, myelin mapping, and T1 relaxometry in the different lesion types




PRLs and the inhomogeneous hyperintense lesions were closer to ventricular CSF than hypo-isointense and homogeneous hyperintense lesions (Table 5).

Table 5. Distances (mean +/- standard deviation) to ventricular CSF and (in colored background) p-values of differences between mean distances in different lesion types. In bold statistically significant p-value (<0.05)

| Lesion QSM susceptibility | Disk distance, mean (SD) | Iso/hypointense | Homogeneous hyperintense | Inhomogeneous hyperintense | PLRs |
|---|---|---|---|---|---|
| Iso/hypointense | 14.8 (+/- 0.5) | - | 0.516 | **<0.001** | **0.017** |
| Homogeneous hyperintense | 14.3 (+/- 0.7) | 0.516 | - | **0.013** | **0.050** |

| | | | | | |
|---|---|---|---|---|---|
| In-homogeneous Hyperintense | 11.9 (+/- 0.7) | **<0.001** | **0.013** | - | 0.649 |
| PRLs | 11.1 (+/-1.5) | **0.017** | **0.050** | 0.649 | - |

*Discussion*

We identified four MS lesion types according to QSM intensity and pattern. Using multimodal qMRI, we found a worsening gradient (hypo-isointense lesions → homogeneous hyperintense lesions → inhomogeneous hyperintense lesions → PRLs) in terms of axonal and myelin damage. These results confirm and extend data from a recent QSM study (Rahmanzadeh et al., 2021), showing that PRLs represent the most destructive MS lesion type, while isointense lesions the most preserved ones. In particular, the T1 relaxometry analysis shows that isointense QSM lesions have shorter T1 values compared to hyperintense lesions, and this is line with the finding that short T1 values are associated with remyelination within MS lesions (Kolb et al., 2021). Altogether these data suggest that QSM isointense lesions could be an optimal biomarker of remyelinated MS lesions in vivo. Such an in vivo biomarker, which can be easily implemented in MRI protocols in clinical trials, might play an important role in assessing the remyelinating potential of newer drugs.

Our results suggest that homogenous hyperintense/inhomogeneous hyperintense/PRLs represent a continuum in the evolution of a MS lesion over time. Homogeneous and inhomogeneous hyperintense lesions may indeed reflect a dynamic process taking place at an earlier stage of the lesion evolution, with the paramagnetic rim appearing lately in a minority of lesions. This hypothesis is line with a recent PET-MRI study using 18F]-DPA-714 (Hamzaoui M et al. ECTRIMS 2022), which showed that more than half of MS lesions have a smoldering component, a frequency much higher than that related to PRLs alone. At post-mortem histology, up to 21% of homogenously hyperintense lesions on QSM are indeed chronic active lesions (Rahmanzadeh et al., 2022). It is therefore likely that PRLs represent only the tip of the iceberg of the CNS smoldering inflammatory component, with the entire spectrum of QSM hyperintense MS lesions representing a continuum of chronic inflammation. Iron and myelin are the main contributors of magnetic susceptibility in white matter. While iron is a paramagnetic material with positive susceptibility with respect to water, myelin is diamagnetic and has negative susceptibility. Accordingly, hyperintensity within lesions, either diffuse or restricted to the lesion borders, is majorly related to the accumulation of iron, rather than

demyelination. It is important to note here that MS lesions often show areas of punctuate hyperintensities in QSM, which most likely correspond to vessels containing deoxygenated hemoglobin. In our cohort, these punctate hyperintensities were frequently observed but not considered as a factor influencing the lesion classification. However, we cannot totally exclude that some inhomogeneous hyperintense lesions have been misclassified because of the presence of several punctuate hyperintensities due to complex vascularization within lesions.

However, controversies on the real biological significance of homogenous and inhomogeneous hyperintense QSM lesions exist. Some studies showed that hyperintense QSM lesions with high punctate or homogenous signal contain no or minimally activated iron myeloid cells (Gillen et al., 2021) and rarely tend to become PRLs (Blindenbacher et al., 2020). Iron in the brain parenchyma is not present exclusively in activated pro-inflammatory microglia. Oligodendrocytes are by far the predominant iron-containing cells as they carry iron-requiring enzymes necessary for myelin production. Intra-lesional iron deposits may thus be the consequence of dying oligodendrocytes releasing iron into the extracellular space, or, on the opposite reflect an increased iron-demand of oligodendrocytes during re-myelination. Another idea is that intra-lesional iron deposits derive from leaky blood vessels within the MS lesions as a consequence of blood brain barrier breakdown. Although histological validation of homogenous and inhomogeneous hyperintense MS lesions is warranted, our study suggest that they could represent a continuum in the evolution of MS pathology.

Which factors promote the evolution of a MS lesion into a chronic active state (i.e., from homogenous hyperintense to PRLs) are still unknown. Given that in our study PRLs were more frequent in the periventricular area, it is tempting to speculate that a noxious activity of CSF, which has been reported by several authors (Magliozzi et al., 2022; Poirion et al., 2021; Tonietto et al., 2022), might drive progression of a MS lesions to a chronic inflammatory state and eventually to a PRL. However, longitudinal data are needed to evaluate this hypothesis.

## 3. SIDE PROJECTS

## Contribution Of Grey Matter Demyelination to Neuronal Damage Evaluated With 11C-flumazenil Positron Emission Tomography In Multiple Sclerosis

Italian Multiple Sclerosis Foundation (FISM) financed research fellowship (Grant 2019/BR/016) at the Paris Brain Institute, Sorbonne University (Mentors: Prof. Bruno Stankoff, Prof. Benedetta Bodini)

Background and Aims

Grey matter (GM) damage is a key contributor of physical and cognitive disability in MS. Despite its clinical relevance, the underlying pathological substrate is still largely unknown. Several pathological mechanisms have been described, including acute axonal transection in inflammatory lesions, energy and metabolic deficits, cortical demyelination, and meningeal inflammation(Brownlee et al., 2019b; Harrison et al., 2017; Magliozzi et al., 2018; Mainero et al., 2015; Zivadinov et al., 2017). Of interest, to which extent grey matter atrophy is driven by subtle inflammatory activity in the cortex is still a matter of debate. MRI techniques alone provide little value in this setting, being unable to distinguish in vivo neurodegenerative and inflammatory mechanisms in the GM, thus providing inaccurate surrogates of neural damage. Against this background, $^{11}$C-flumazenil positron emission tomography (PET) is a promising technique which has proven to be a sensitive quantitative marker to map the neuronal substrate of GM pathology in MS (Freeman et al., 2015). The validation of such a quantitative and specific marker of neural damage would allow to predict people with MS (PwMS) with a higher risk of developing sustained physical and cognitive disability, who thus might benefit from early aggressive treatment. Moreover, the combined use of $^{11}$C-flumazenil PET with MRI has the potential to disentangle the relative contribution of neurodegeneration and demyelination in the GM of MS patients, eventually promoting the development of new neuroprotective drugs. A combined high field MRI and $^{11}$C-flumazenil PET study is ongoing at the Paris Brain Institute (Sorbonne University), with the aim to assess neuronal damage in the early phase of either relapsing or progressive MS. The main objective of the study is to robustly quantify and map $^{11}$C-flumazenil binding changes in the GM of MS subject with different clinical phenotypes, compared to healthy controls and to assess the relations between PET findings and clinical disability, at both an individual and group level. Other exploratory studies are to assess the contribution of focal and diffuse GM

demyelination (focal cortical lesions and diffuse cortical demyelination) on neuronal damage evaluated with $^{11}$C-flumazenil PET.

## Methods

### *Study population*

We used MRI and clinical data from two longitudinal prospective studies (*NCT02305264, NCT01651520*) conducted at the Paris Brain Institute, Sorbonne University, from 2014 to 2021. Both study protocols included a baseline 3T MRI scan for PwMS and healthy controls (HC) and a 12-month follow-up scan for PwMS only. Complete neurological assessment and a similar neuropsychological test battery was performed at both timepoints. Detailed inclusion/exclusion criteria and study protocols are reported at www.clinicaltrials.gov.

### *Clinical and neuropsychological evaluation*

PwMS underwent full baseline neurological examination with Expanded-Disability-Status-Scale (EDSS) and Multiple-Sclerosis-Severity-Scale (MSSS) scoring. Subjects underwent 9-Hole Peg Test (9HPT), Timed-25-Foot-Walk (T25FW), Paced Auditory Serial Addition Test (PASAT) and Symbol-Digit-Modalities-Test (SDMT). Baseline neuropsychological examination also included: Hospital Anxiety and Depression Scale (HADS), Modified Fatigue Impact Scale (MFIS), phonemic (Controlled-oral-word-association-test, COWAT) and semantic (Semantic-verbal-fluency, SVF) fluency tests and verbal memory span test (Forward-digit-span, FDS). PwMS were re-evaluated after 12 months with the same neurological assessment. Z-scores were calculated for each test, and z-scores <-1 were considered altered.

### *Image acquisition*

Subjects were scanned with an identical MRI protocol using 2 different Siemens MRI scans (Prisma and Trio) at the Paris Brain Institute. The MRI protocol included the following sequences: 3D-T1-weighted magnetization-prepared-rapid-gradient-echo (MPRAGE, TR/TE 2300/2.98 ms, inversion time 900 ms, resolution 1x1x1 mm3), T2-weighted images (TR/TE 4000/83 ms, resolution 0.9x0.9x3 mm3), 3D fluid-attenuation-inversion-recovery (FLAIR, TR/TE 8880/129 ms, inversion time 2500 ms, resolution 0.9x0.9x3 mm3), 3D gradient-echo with (MTon) and without (MToff) magnetization transfer (TR/TE 35/5 ms, resolution 1x1x2 mm3) and resting state echo-planar imaging (200

volumes, TR/TE 2100/25 ms, resolution 1x1x2 mm3). The NCT01651520 protocol also included double inversion recovery (DIR) images (TR/TE 8000/253, 1x1x1 mm3)

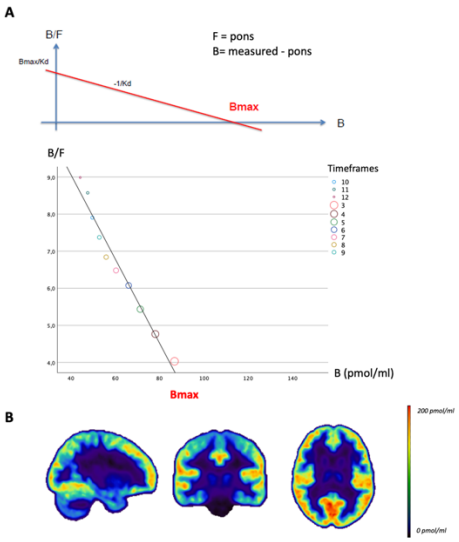
$^{11}$C-FMZ PET was available for 41 subjects enrolled in the NCT01651520 trial. All subjects underwent PET on a High-Resolution Research Positron Tomograph (HRRT; spatial resolution 5 2.5mm; Siemens Medical Solutions, Knoxville, TN) at Service Hospitalier Frederic Joliot, CEA.

*PET analyses*

$^{11}$C-FMZ PET was acquired using a partial saturation protocol, as described in Delforge et al(Delforge et al., 1997). Ready-to-inject, >99% radiochemically pure $^{11}$C-FMZ was prepared from cyclotron-produced $^{11}$C-carbon dioxide using a commercially available TRACERLab FX-C synthesizer (General Electric Medical Systems). Protocol consisted of a coinjection of $^{11}$C-labeled FMZ and unlabeled FMZ (10 mg/kg, Anexate) to partially saturate the GABA-A receptors.

The in vivo quantification of the benzodiazepine receptor concentration in humans using FMZ-PET is usually based on Scatchard analysis when the goal is to avoid blood sampling. The experimental protocol, however, necessarily requires several (at least two) experiments with various specific activities in the same subject to obtain a range of bound ligand concentrations. The partial saturation protocol with unlabeled FMZ, which is based on the natural decrease in bound ligand concentration after an FMZ injection with an average dose between a tracer dose and a saturation dose, provides instead an adequate range of bound ligand concentrations, allowing the simultaneous estimation of Bmax and Kd in a single PET experiment. The free ligand concentration is estimated from the PET measurement in the pons after correction for the effect of the small receptor site concentration in this reference region. Figure 1 shows the Scatchard analysis for the occipital cortex of a single subject, with Bmax and Kd estimation.

*Figure 1: Panel A shows the Scatchard analysis with the FMZ partial saturation protocol. Panel B shows the parametric estimation of brain GABA-A concentration in 10 HC.*




For 6 HC, test-retest $^{11}$C-FMZ PET scans were available. Except for one patient, for whom Scatchard analysis was not reliable due to abnormal partial saturation of GABA-A receptor in the second scan, test-rest was good, with a maximum variability of Bmax estimation in whole cortical grey matter of 6.5%.

*MRI analyses*

Careful visual inspection of all MRI images was performed to ensure high image quality. Due to low quality of 3D-T1 images, 1 scan was excluded. For 20 MRI scans, MTI was not available and for 9 was of insufficient quality (due to excessive motion and/or incomplete field-of-view). Resting-fMRI was not available in 10 MRI scans and for 15 was of suboptimal quality due to excessive motion (according to the scrubbing threshold adopted during image processing -see Resting-state functional MRI section-).

T1 and T2 weighted imaging

In PwMS, MS lesions were manually segmented at each timepoint on T2-FLAIR images using Jim v.6.0 (Xinapse systems, Essex-UK, http://www.xinapse.com/). T2-FLAIR images at each timepoint were rigidly aligned to the corresponding 3D-T1 images using Advanced Normalization Tools (ANTs) 2.3 (http://stnava.github.io/ANTs/). After lesion-filling of the corresponding 3D-T1 images, total brain, grey matter, white matter, and cerebrospinal fluid were segmented in all patients using CAT12, part of SPM12 (http://www.neuro.uni-jena.de/cat/). Moreover, cortical lobar GM

segmentation according to the Desikan-Killiany atlas was obtained using FreeSurfer (https://surfer.nmr.mgh.harvard.edu/). All images were visually checked and manually edited if necessary. 3D-T1 images were then normalized to the MNI152_nlin_asym_09c standard space using a nonlinear registration with ANTs. The derived transformations were combined to register all other images (MTR, resting fMRI and segmented regions) from native space to MNI. For longitudinal analyses, a within-subject, half-way space was created for each subject. This approach reduces the bias introduced by interpolation if the first MRI scan is used as the reference scan, allowing to treat each MRI timepoint equally.

Magnetization transfer imaging

In all subjects, MTR maps were calculated in the native space using the formula MTR=(MToff-MTon)/MToff. Patient-specific maps of cortical demyelination were computed at each timepoint in the MNI space as described in Lazzarotto et al(Lazzarotto et al., 2022). Briefly, to classify a cortical voxel as "demyelinated" we employed a threshold technique using 84 HC as a reference group (Lazzarotto et al. manuscript in preparation, presented at ECTRIMS 2021), using a voxel-wise permutation-based two-sample t-test adjusted for age and sex, thresholded at $p<0.05$, after threshold-free cluster enhancement correction. In these significant regions, we computed the MTR mean relative difference between patients and healthy controls [(mean MTR in healthy controls – mean MTR in patients)/mean MTR in healthy controls]. In each patient at each time-point, we then calculated the relative difference between the MTR value of any given cortical voxel and the average MTR value of all voxels in healthy controls of the same center localized at the same distance than the given voxel from the CSF (thus suffering from the same degree of partial volume effect). If this difference was greater than the previously calculated threshold, that cortical voxel was classified as "demyelinated", otherwise as "normally myelinated".

Resting state functional MRI

Resting-state echo-planar images were processed using SPM12. Processing included realignment, slice timing correction, low-pass filtering (0.01-0.1 Hz), spatial smoothing (2.5x2.5x3), normalization to SPM specific standard-space and nuisance signal regression of the 6 motion parameters obtained from realignment. To further reduce motion artefacts, we performed a conservative scrubbing of corrupted frames (defined as frames with a displacement >0.5, as described in Power et al.(Power et al., 2012)) and we removed all subjects presenting >25% of displaced frames. To reduce noise

coming from signal distortions at air-tissue interfaces in the frontal and temporal poles, we removed voxels with an outlier value in BOLD signal (defined as BOLD values in the first and last 0.5th percentile of each scan). Functional connectivity density mapping, an ultrafast graph theory for quantifying local connectivity metric at the voxel level without the need of any hypothesis-driven seed-based correlation approach, was then performed for each subject. Specifically, we calculated the local functional connectivity density (lFCD) index, defined as the number of voxels that correlate (Pearson r>0.6) with all the voxels situated within 12 mm distance in any direction (https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dLFCD.html), representing local neuronal hubness.

Double inversion recovery

DIR images were registered to the corresponding 3D-T1 and FLAIR images using Ants. According to published guidelines(Geurts et al., 2011), cortical lesions that appear as focal cortical hyperintensities and extend for at least 3 voxels and across 2 consecutive slices, have been segmented with Jim v.6.0 (Xinapse systems, Essex-UK, http://www.xinapse.com/). Cortical lesion mask has been registered to $^{11}$C-FMZ-PET and intersected with the mask of the cortex, to retain in the analyses only the intra-cortical portion of the cortical lesions.

*Statistical analyses*

Statistical analyses were performed using SPSS (v.28 IBM Statistics) and R (https://www.r-project.org/). Two-sided p-values<0.05 were considered significant. Descriptive results were reported as mean with standard deviation (SD) or median with interquartile range (IQR) or range. Shapiro–Wilk test and visual inspection of the histograms were performed to evaluate variable distribution. To consider the effect of scanner variability within the study, all MRI analyses were adjusted for MRI scan. Differences in clinical and radiological variables between study sub-groups were assessed with the Wilcoxon test, Kruskal-Wallis test and multivariate generalized linear model with Tukey's post-hoc test, as appropriate. The association between clinical variables and brain volumes and MRI markers of cortical neuronal density, myelin content and functional connectivity was investigated with Spearman's rank correlation. Linear and binary logistic regression models were used to explore the contribution of different PET-MRI metrics on clinical and cognitive status. Results were reported as beta coefficient or odds-ratio (OR) with the corresponding 95% confidence interval (CI). Variables significantly associated with each outcome on univariate analysis were

included as covariates in the multivariate model, reporting global R2 with individual standardized beta coefficients or OR.

Voxelwise and region-of-interest analyses

For voxel-wise analyses, permutation-based two-sample t-test using a threshold-free cluster enhancement correction (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise) was performed to assess voxelwise differences in PET-MRI markers of cortical pathology between study sub-groups. To explore the spatial relationship between cortical neuronal density and lFCD, we employed VoxelStats, a MATLAB package which allows multi-modal, voxel-wise brain analyses (https://github.com/sulantha2006/VoxelStats).

Surface-based comparisons were performed using a general linear model to test Bmax signal differences between study subgroups on a vertex-by-vertex basis, adjusting for age and sex (using the mris_preproc, mri_surf2surf and mri_glmfit command from FreeSurfer, https://surfer.nmr.mgh.harvard.edu/fswiki/MultiModalTutorialV6.0/FMRIGroupAnalysis). A statistical threshold of $p < 0.05$ with false-discovery rate correction was used.

To further explore the effect of cortical myelin content and longitudinal demyelination/remyelination on GABA-A receptor density, we performed a region-of-interest (ROI) analysis using an individualized cortical parcellation approach. In detail, baseline cortical demyelinated voxels were clustered within the cortex mask in each subject, to generate myelin specific cortical parcellations, which were then classified as presenting mild (<2%), moderate (2-11%) or severe baseline demyelination (>11%), according to their percentage of demyelinated voxels. The thresholds were chosen according to the 25th and 75th percentile values of the entire cohort of patients. To account for the lack of statistical independence among results for cortical parcellations within the same patient, an anonymized subject ID was incorporated into each model.

## Results

Table 1 and 2 reports the clinical and demographic characteristics of the study cohorts.

*Table 1. $^{11}$C-FMZ PET cohort*

| | PwMS (31) | HC (10) |
|---|---|---|

| | | |
|---|---|---|
| Age, y (mean±SD) | 41.2 (11.6) | 35.5 (9.3) |
| Females, female n (%) | 13 (43) | 60 (6) |
| Progressive MS n (%) | 31 (74.6) | - |
| Disease duration, y (mean±SD) | 3.6 (2.9) | - |
| EDSS, median (IQR) | 2 (1-3) | - |
| PASAT, (mean±SD) | 45.9 (11.1) | 50.8 (7.8) |
| Brain volume, ml (mean±SD) | 1154.4 (139.8) | 1112.6 (129.2) |
| Cortical volume, ml (mean±SD) | 441.7 (51.2) | 460.1 (44.3) |
| T2 lesion load, ml (mean±SD) | 7.5 (7.5) | - |

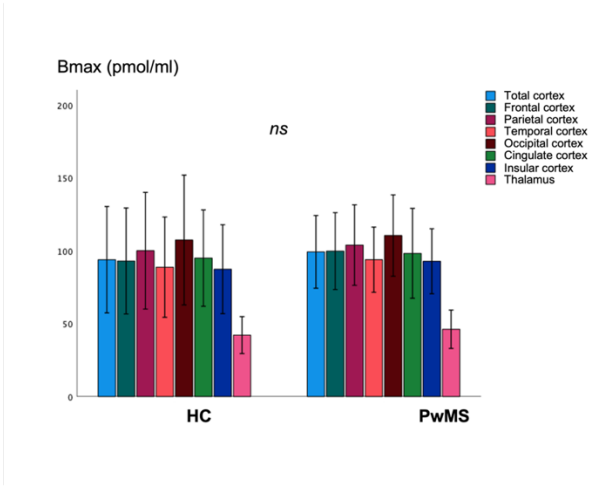
*Table 2. Resting state and MTR cohort*

| | PwMS (48) |
|---|---|
| Age, y (mean±SD) | 47.5 (11.8) |
| Females, female n (%) | 29 (60.4) |
| Progressive MS n (%) | 31 (74.6) |
| Disease duration, y (mean±SD) | 7.6 (5.6) |
| EDSS, median (IQR) | 4 (3-6) |
| PASAT, (mean±SD) | 40.0 (12.7) |
| Brain volume, ml (mean±SD) | 1087.2 (105.7) |
| Cortical volume, ml (mean±SD) | 441.7 (51.2) |
| T2 lesion load, ml (mean±SD) | 11.8 (8.6) |
| Cortical demyelination, % (mean±SD) | 5.5 (6.5) |
| Total lFCD, (log mean±SD) | 0.14 (0.12) |

As reported in Figure 2., no differences were detected in GABA-A receptor density (Bmax) between PwMS and HC in the total cerebral cortex, the deep grey matter nuclei, and the individual cortical lobes. Similarly, the voxel-wise parametric analysis and the surface-based analysis did not show

areas of different neuronal density between the two groups (data not shown). No differences were noted between patients with relapsing-remitting MS and progressive MS.

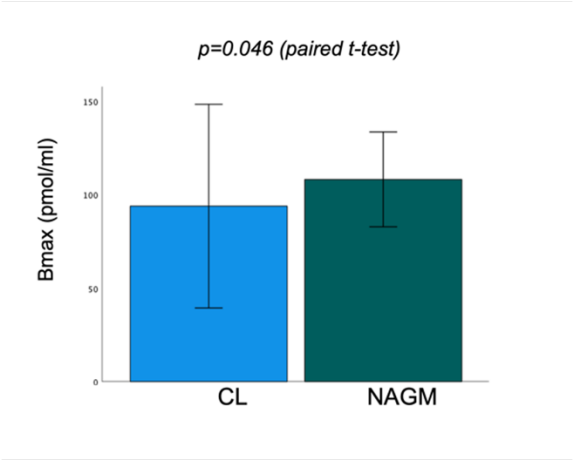
*Figure 2. Comparison of GABA-A receptor densities between PwMS and HC*




A significant inverse correlation was found between higher MSSS and lower occipital Bmax (p=0.44, rho=-0.42), along with a trend towards higher EDSS and lower occipital Bmax (p=0.057, rho=-0.39). These two results did not survive correction for multiple comparisons. No correlations were found between cortical and subcortical Bmax values and neuropsychological scores.

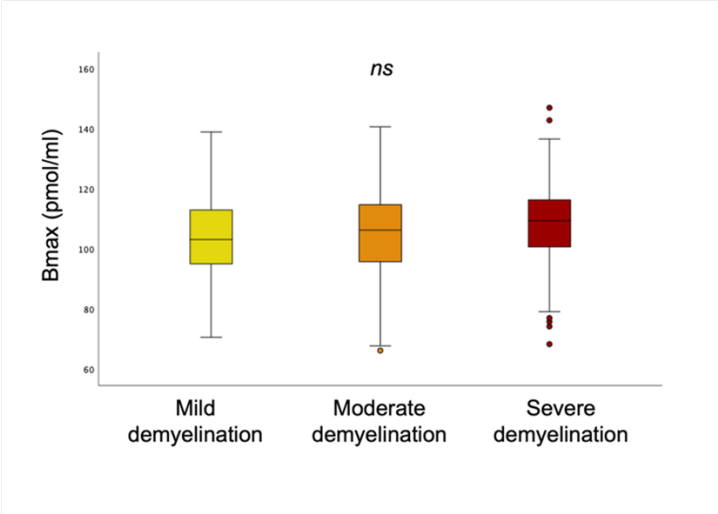
We found that in PwMS, neuronal density within cortical GM lesions was significantly inferior compared to the corresponding normal appearing GM (Figure 3.). A high heterogeneity was found within cortical lesions, with some lesions showing Bmax values in the range of the cortex of the HCs.

Figure 3. *Comparison of GABA-A receptor densities in cortical lesions (CL) and normal-appearing grey matter (NAGM) in PwMS.*



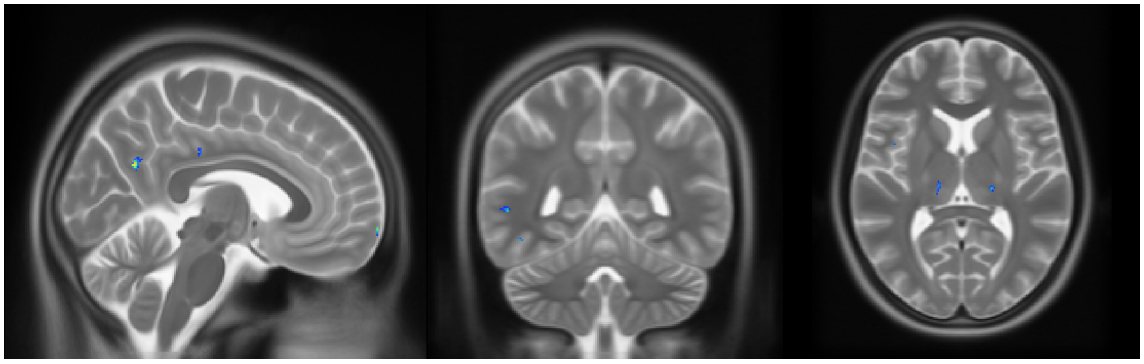

Of note, no correlations were found between intracranial-volume-corrected cortical volumes and Bmax. No differences were noted in Bmax values in “demyelinated” vs “normally myelinated” cortical voxels. Similarly, cortical parcellations had similar mean Bmax values independently of their degree of demyelination (Figure 4.).

*Figure 4. GABA-A receptor densities values according to the level of cortical demyelination.*



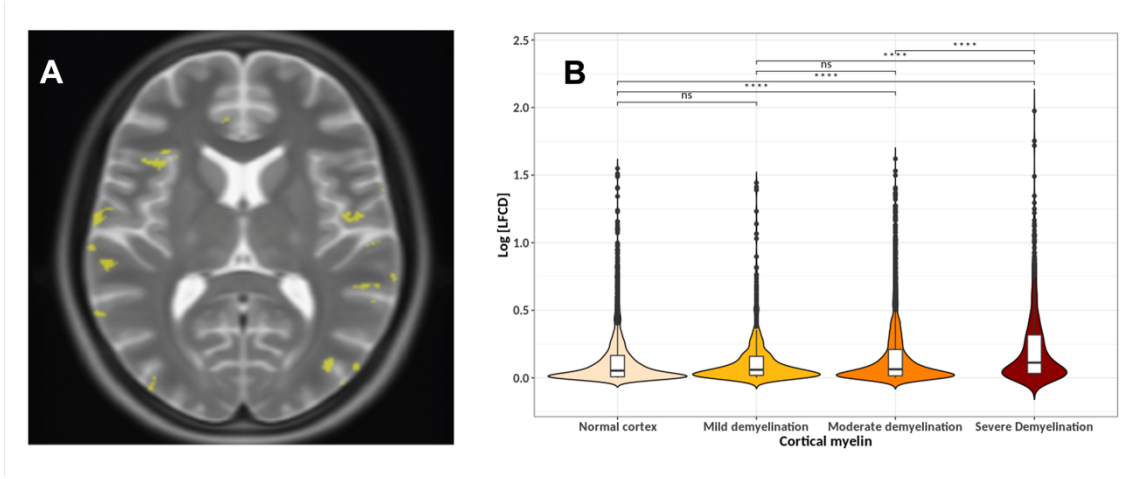

To explore the spatial association between GABA-A receptor density and lFCD, we performed a multimodal voxel wise analysis (Figure 5.) which revealed some cluster of positive relationship between FMZ and LFCD in the precuneus, posterior cingulate, thalami and parieto-temporal cortex (family-wise-error-corrected, $p<0.05$).

*Figure 5. Multimodal voxel-wise analysis between cortical GABA-A receptor concentration and local neuronal connectivity density.*



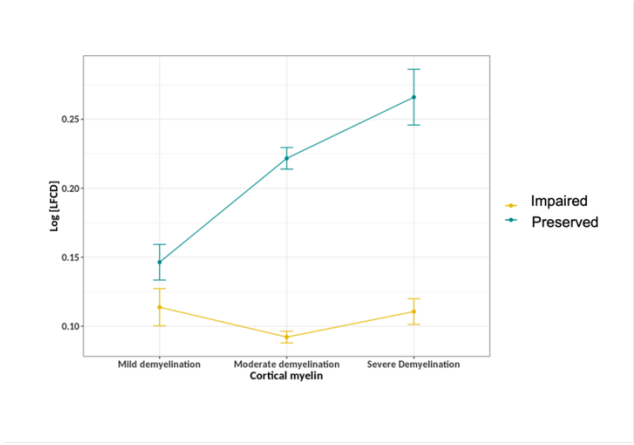
Given the relationship between GABA-A density and lFCD, we analyzed the changes in lFCD associated with different degree of demyelination/remyelination, with the aim at characterizing the interplay between these processes on cognitive functioning. Using multimodal parametric analysis, we found that in PwMS, decreased myelin content was associated with significantly higher LFCD, reflecting enhanced neuronal local connectivity (Figure 6.)

*Figure 6. Panel A reports the multimodal voxel-wise analysis between cortical MTR and LFCD showing significant inverse relationship between MTR and LFCD. Panel B reports the ROI analysis of LFCD values according to the level of cortical demyelination.*



For the same extent of cortical demyelination, lFCD enhancement was very heterogenous among patients, with cognitively preserved PwMS showing a significantly higher compensatory lFCD than cognitively impaired PwMS (Figure 7.; p<0.001, Partial-Eta-Squared=0.47).

*Figure 7. Comparison between patients with pathological PASAT z-scores and patients with preserved PASAT z-scores (Error bars showing the 95% standard error).*




Cognitive preserved PwMS with high compensatory neuronal local connectivity were younger (p<0.0001; Partial Eta Squared 0.07) and had a lower white matter lesion burden (p<0.0001; Partial Eta Squared 0.25) than cognitive impaired PwMS with limited lFCD enhancement.
Over 12 months, neuronal connectivity enhancement was shown to fade off in the presence of excessive dynamic cortical demyelination (>12%). Conversely, greater cortical remyelination was associated by higher LFCD enhancement (p<0.001, Partial-Eta-Squared=0.19) (data not shown).

Discussion

Using $^{11}$C-flumazenil PET with a partial saturation protocol, we were able to quantify the density of GABA-A receptors in 10 healthy controls (HC) and 31 people with multiple sclerosis (PwMS). In line with some ("ECTRIMS 2021 – Late Breaking News Oral Presentations," 2021; Kang et al., 2020), but not all (Freeman et al., 2015) works, we did not find significant decrease in grey matter neuronal density in PwMS compared to HC. These contradictory *in vivo* results are paralleled by post-mortem studies (Huiskamp et al., 2022; Preziosa et al., 2019; Vercellino et al., 2022), in which the amount of cortical neuronal loss varies greatly from one report to another. Our results have different

explanations. First, cortical atrophy, which is routinely seen in PwMS even in the early stages of the disease, could be a consequence of several pathological mechanisms other than pure neuronal loss, such as the reduction of the extracellular matrix and/or the reduction of astrocytes and oligodendrocytes. Second, it is possible that in early MS an upregulation of post-synaptic GABA-A receptors takes place along the dendrites and the neuronal soma, hindering the reduction of total cortical neurons. Other groups have indeed reported increased $^{11}$C-FMZ in PwMS compared to HC ("ECTRIMS 2021 – Late Breaking News Oral Presentations," 2021; Kang et al., 2020), which in one study was associated with increased microglia activation(Kang et al., 2020), raising the possibility that diffuse cortical inflammatory processes can induce an up-regulation of post-synaptic GABA-A receptors. Conversely to our previous work(Freeman et al., 2015), which was composed by PwMS with a longer disease duration, the disease duration of this study cohort was quite short (mean of 3 years). It is therefore tempting to speculate that in early MS there is a compensatory increase in GABA-A receptors, which fades away in the advanced phases of the disease. This hypothesis largely resembles the thesis of networks adaptative/maladaptive remodeling, which is well-known in functional connectivity studies. In line with this hypothesis, we found that GABA-A receptor density has a similar topography to that of measures of neuronal functional connectivity, such as local functional connectivity density. Interestingly, our preliminary analysis on the relationship between cortical pathology and functional reorganization showed that decreased myelin content was associated with significantly higher local functional connectivity density, with higher levels associated with better cognitive outcomes. Globally, these results highlight the close interaction between cortical damage in terms of neuronal loss and demyelination and functional adaptive changes.

Lastly, we found that, although cortical lesions have less neuronal density than the corresponding normal appearing grey matter on a global level, a great heterogeneity in GABA-A receptor density exists within different cortical lesions. It is well established that white matter lesions are heterogeneous in terms of inflammatory infiltrates(Bodini et al., 2020) and myelin content(Bodini et al., 2016), and this could be the case also for cortical lesions, which could present different degrees of neuronal loss.

## 4. PUBLICATIONS

**Articles published during PhD (From 01/11/2019 to 01/01/2023):**

Long-Term Clinical Outcomes of Hematopoietic Stem Cell Transplantation in Multiple Sclerosis.

**Boffa G**, Massacesi L, Inglese M, Mariottini A, Capobianco M, Lucia M, Amato MP, Cottone S, Gualandi F, De Gobbi M, Greco R, Scimè R, Frau J, Zimatore GB, Bertolotto A, Comi G, Uccelli A, Signori A, Angelucci E, Innocenti C, Ciceri F, Repice AM, Sormani MP, Saccardi R, Mancardi G; Italian BMT-MS study group.

*Neurology. 2021 Jan 20:10.1212/WNL.0000000000011461. doi: 10.1212/WNL.0000000000011461.*

Predictors of Ocrelizumab Effectiveness in Patients with Multiple Sclerosis

Cellerino M*, **Boffa G***, Lapucci C, Tazza F, Sbragia E, Mancuso E, Bruschi N, Minguzzi S, Ivaldi F, Poirè I, Laroni A, Mancardi G, Capello E, Uccelli A, Novi G, Inglese M.

*Neurotherapeutics. 2021 Sep 22:1-10. doi: 10.1007/s13311-021-01104-8.*

Hematopoietic Stem Cell Transplantation In People With Active Secondary Progressive Multiple Sclerosis

**Boffa G***, Signori A*, Massacesi L, Mariottini A, Sbragia E, Cottone S, Amato MP, Gasperini C, Moiola L, Meletti S, Sbragia E, Repice AM, Brescia Morra V, Salemi G, Patti F, Filippi M, De Luca G MD, Lus G MD, Zaffaroni M, Sola P, Conte A, Nistri R, Aguglia U, Granella F, Galgani S, Caniatti LM, Lugaresi A, Romano S, Iaffaldano P, Cocco E, Saccardi R, Angelucci E, Trojano M, Mancardi GL, Sormani MP and Inglese M on behalf of the Italian BMT-MS Study Group and the Italian MS Register

*Neurology. 2022 Dec 21:10.1212/WNL.0000000000206750. doi: 10.1212/WNL.0000000000206750*

Personalizing ocrelizumab treatment in Multiple Sclerosis: What can we learn from Sars-Cov2 pandemic?

Tazza F, Lapucci C, Cellerino M, **Boffa G**, Novi G, Poire I, Mancuso E, Bruschi N, Sbragia E, Laroni A, Capello E, Inglese M.

*J Neurol Sci. 2021 Aug 15;427:117501. doi: 10.1016/j.jns.2021.117501.*

Treatment of multiple sclerosis with rituximab: A multicentric Italian-Swiss experience.

Zecca C, Bovis F, Novi G, Capobianco M, Lanzillo R, Frau J, Repice AM, Hakiki B, Realmuto S, Bonavita S, Curti E, Brambilla L, Mataluni G, Cavalla P, Di Sapio A, Signoriello E, Barone S, Maniscalco GT, Maietta I, Maraffi I, **Boffa G**, Malucchi S, Nozzolillo A, Coghe G, Mechi C, Salemi G, Gallo A, Sacco R, Cellerino M, Malentacchi M, De Angelis M, Lorefice L, Magnani E, Prestipino E, Sperli F, Brescia Morra V, Fenu G, Barilaro A, Abbadessa G, Signori A, Granella F, Amato MP, Uccelli A, Gobbi C, Sormani MP.

*Mult Scler. 2020 Oct;26(12):1519-1531. doi: 10.1177/1352458519872889.*

Impact of treatment on cellular immunophenotype in MS: A cross-sectional study.

Cellerino M, Ivaldi F, Pardini M, Rotta G, Vila G, Bäcker-Koduah P, Berge T, Laroni A, Lapucci C, Novi G, **Boffa G**, Sbragia E, Palmeri S, Asseyer S, Høgestøl E, Campi C, Piana M, Inglese M, Paul F, Harbo HF, Villoslada P, Kerlero de Rosbo N, Uccelli A.

*Neurol Neuroimmunol Neuroinflamm. 2020 Mar 5;7(3):e693. doi: 10.1212/NXI.0000000000000693.*

Vaccinations in patients with multiple sclerosis: a real-world, single-center experience.

Sbragia E, Olobardi D, Novi G, Lapucci C, Cellerino M, **Boffa G**, Laroni A, Mikulska M, Sticchi L, Inglese M.

*Hum Vaccin Immunother. 2022 Nov 30;18(6):2099171. doi: 10.1080/21645515.2022.2099171.*

What happens after fingolimod discontinuation? A multicentre real-life experience.

Landi D, Signori A, Cellerino M, Fenu G, Nicoletti CG, Ponzano M, Mancuso E, Fronza M, Ricchiuto ME, **Boffa G**, Inglese M, Marfia GA, Cocco E, Frau J.

*J Neurol. 2022 Feb;269(2):796-804. doi: 10.1007/s00415-021-10658-8. Epub 2021 Jun 16.*

Embracing resilience in multiple sclerosis: a new perspective from COVID-19 pandemic.

Sbragia E, Colombo E, Pollio C, Cellerino M, Lapucci C, Inglese M, Mancardi G, **Boffa G**.

*Psychol Health Med. 2022 Feb;27(2):352-360. doi: 10.1080/13548506.2021.1916964.*

Physical Exercise Moderates the Effects of Disability on Depression in People with Multiple Sclerosis during the COVID-19 Outbreak.

Carotenuto A, Scandurra C, Costabile T, Lavorgna L, Borriello G, Moiola L, Inglese M, Trojsi F, Petruzzo M, Ianniello A, Nozzolillo A, Cellerino M, **Boffa G**, Rosa L, Chiodi A, Servillo G, Moccia M, Bonavita S, Filippi M, Petracca M, Brescia Morra V, Lanzillo R.
*J Clin Med. 2021 Mar 16;10(6):1234. doi: 10.3390/jcm10061234.*

Menstrual cycle resumption and female fertility after autologous hematopoietic stem cell transplantation for multiple sclerosis.
Massarotti C, Sbragia E, **Boffa G**, Vercelli C, Zimatore GB, Cottone S, Frau J, Raiola A, Varaldo R, Mancardi G, Inglese M, Anserini P.
*Mult Scler. 2021 Nov;27(13):2103-2107. doi: 10.1177/13524585211000616.*

Relationship Between Retinal Layer Thickness and Disability Worsening in Relapsing-Remitting and Progressive Multiple Sclerosis.
Cellerino M, Priano L, Bruschi N, **Boffa G**, Petracca M, Novi G, Lapucci C, Sbragia E, Uccelli A, Inglese M.
*J Neuroophthalmol. 2021 Sep 1;41(3):329-334. doi: 10.1097/WNO.0000000000001165.*

Ultra-high-field 7-T MRI in multiple sclerosis and other demyelinating diseases: from pathology to clinical practice.
Bruschi N, **Boffa G**, Inglese M.
*Eur Radiol Exp. 2020 Oct 22;4(1):59. doi: 10.1186/s41747-020-00186-x*

COVID-19 pandemic and mental distress in multiple sclerosis: Implications for clinical management.
Costabile T, Carotenuto A, Lavorgna L, Borriello G, Moiola L, Inglese M, Petruzzo M, Trojsi F, Ianniello A, Nozzolillo A, Cellerino M, **Boffa G**, Rosa L, Servillo G, Moccia M, Bonavita S, Filippi M, Lanzillo R, Brescia Morra V, Petracca M.
*Eur J Neurol. 2021 Oct;28(10):3375-3383. doi: 10.1111/ene.14580.*

Degree of microstructural changes within T1-SE versus T1-GE hypointense lesions in multiple sclerosis: relevance for the definition of "black holes".
Lapucci C, Romano N, Schiavi S, Saitta L, Uccelli A, **Boffa G**, Pardini M, Signori A, Castellan L, Inglese M, Roccatagliata L.

*Eur Radiol. 2020 Jul;30(7):3843-3851. doi: 10.1007/s00330-020-06761-5.*

Fingolimod and Dimethyl-Fumarate-Derived Lymphopenia is not Associated with Short-Term Treatment Response and Risk of Infections in a Real-Life MS Population.

**Boffa G**, Bruschi N, Cellerino M, Lapucci C, Novi G, Sbragia E, Capello E, Uccelli A, Inglese M.

*CNS Drugs. 2020 Apr;34(4):425-432. doi: 10.1007/s40263-020-00714-8.*

Aggressive multiple sclerosis: a single-centre, real-world treatment experience with autologous haematopoietic stem cell transplantation and alemtuzumab.

**Boffa G**, Lapucci C, Sbragia E, Varaldo R, Raiola AM, Currò D, Roccatagliata L, Capello E, Laroni A, Mikulska M, Gualandi F, Uccelli A, Angelucci E, Mancardi GL, Inglese M.

*Eur J Neurol. 2020 Oct;27(10):2047-2055. doi: 10.1111/ene.14324.*

Prevalence of disability improvement as a potential outcome for multiple sclerosis trials.

Signori A, **Boffa G**, Bovis F, Mariottini A, Repice A, Inglese M, Amato MP, Mancardi G, Massacesi L, Saccardi R, Sormani MP.

*Mult Scler. 2021 Apr;27(5):706-711. doi: 10.1177/1352458520936236.*

Tailoring B cell depletion therapy in MS according to memory B cell monitoring.

Novi G, Bovis F, Fabbri S, Tazza F, Gazzola P, Maietta I, Currò D, Bruschi N, Roccatagliata L, **Boffa G**, Lapucci C, Pesce G, Cellerino M, Solaro C, Laroni A, Capello E, Mancardi G, Sormani M, Inglese M, Uccelli A.

*Neurol Neuroimmunol Neuroinflamm. 2020 Aug 4;7(5):e845. doi: 10.1212/NXI.0000000000000845.*

Autologous hematopoietic stem cell transplantation following alemtuzumab therapy in aggressive multiple sclerosis: A report of three cases.

**Boffa G**, Sbragia E, Raiola AM, Varaldo R, Capello E, Gallo P, Granella F, Mancardi G, Inglese M.

*Mult Scler. 2021 Jun;27(7):1145-1148. doi: 10.1177/1352458520914818.*

Severe disease activity in MS patients treated with cladribine after fingolimod withdrawal.

Cellerino M, Bonavita S, Ferrero M, Inglese M, **Boffa G.**

*J Neurol Sci. 2020 Nov 15;418:117156. doi: 10.1016/j.jns.2020.117156.*

Central vein sign and diffusion MRI differentiate microstructural features within white matter lesions of multiple sclerosis patients with comorbidities

Lapucci C, Tazza F, Rebella S, **Boffa G**, Sbragia E, Bruschi N, Mancuso E, Mavilio N, Signori A, Roccatagliata L, Cellerino M, Schiavi S, Inglese M

*Frontiers in Neurology, in press*

**Articles under review:**

PET with [18F]-DPA-714 unveils a smoldering component in most MS lesions

Hamzaoui M, Garcia J, **Boffa G**, Lazzarotto A, Ricigliano V, Soulier T, Tonietto M, Gervais P, Bissery A, Louapre C, Bottlaender M, Benedetta B, Stankoff B

In-vivo characterization of macro- and microstructural injury of the subventricular zone in relapsing-remitting and progressive multiple sclerosis

Maria Cellerino, Simona Schiavi, Caterina Lapucci, Elvira Sbragia, **Giacomo Boffa**, Claudia Rolla-Bigliani, Serena Tonelli, Daniele Boccia, Nicolò Bruschi, Francesco Tazza, Diego Franciotta and Matilde Inglese

Clinical and radiological correlates of apathy in Multiple Sclerosis

Tazza, Francesco; Schiavi, Simona; Colombo, Eleonora; Gualco, Caterina; Cellerino, Maria; **Boffa, Giacomo**; Ballerini, Stefania; Tonelli, Serena; Sbragia, Elvira; Inglese, Matilde; Lapucci, Caterina

**Abstract submitted to scientific congresses during PhD (From 01/11/2019 to 01/01/2023):**

The smoldering component of white matter lesions contributes to disease progression in multiple sclerosis: a longitudinal 18 F-DPA-714 TSPO PET study

Hamzaoui M, Garcia J, **Boffa G**, Lazzarotto A, Ricigliano V.AG, Louapre C, Yazdan-Panah A, Soulier T, Bodini B, Stankoff B

*Oral communication at the Young Scientific Investigator's Session ECTRIMS 2022 and oral communication at ISMRM 2023*

Increase in neuronal local connectivity following cortical demyelination prevents cognitive impairment in multiple sclerosis

**Boffa G**, Hamzaoui M, Lazzarotto A, Ricigliano V.G.A, Dubessy A.L., Shokri-Kojori E, Inglese M, Stankoff B, Bodini B

*Poster presentation at ECTRIMS 2022*

Cortical remyelination predicts cortical atrophy and clinical progression in early multiple sclerosis

Lazzarotto A, HamzaouI M, Tonietto M, Ricigliano V.A.G., **Boffa G**, Khalil M, Pirpamer L, Ropele S, Enzinger C, Battaglini M, Stromillo M.L, De Stefano N, Rocca M.A, Gallo P, Gasperini C, Stankoff B, Bodini

*Oral communication at the Young Scientific Investigator's Session ECTRIMS 2021*

Impact of highly active immunotherapy on acute and chronic neuroinflammation in aggressive multiple sclerosis

**G. Boffa**, S. Schiavi, F. Tazza, E. Sbragia, T. Clementi, C. Lapucci, J. Celada Ballanti, T. Sirito, D. Boccia, M. Cellerino, G. Mancardi, M. Inglese

*Oral communication at SIN 2022*

The effect of cladribine versus fingolimod on clinical and MRI measures in relapsing remitting multiple sclerosis

M. Cellerino , T. Sirito , S. Schiavi , F. Tazza , C. Pierella , D. Boccia , E. Mancuso , **G. Boffa** , E. Capello, C. Lapucci , M. Inglese

*Poster presentation at ECTRIMS 2022*

The effect of ocrelizumab versus oral highly active immunotherapies on white matter microstructure in relapsing remitting multiple sclerosis

C. Lapucci , F. Tazza , C. Pierella , S. Schiavi , D. Boccia , E. Mancuso , T. Sirito , **G. Boffa** , M. Cellerino, M. Inglese

*Poster presentation at ECTRIMS 2022*

Effect of Ocrelizumab treatment on retinal atrophy: a single-center prospective observational study

M. Cellerino , E. Mancuso , **G. Boffa** , N. Bruschi , G. Novi , L. Gaetano , S. Magon , A. Uccelli , C. Lapucci , M. Inglese

*Poster presentation at ECTRIMS 2022*

The role of RIM lesions in predicting longitudinal brain and retinal atrophy

M. Cellerino , F. Tazza , S. Schiavi , C. Pierella , T. Sirito , D. Boccia , E. Mancuso , **G. Boffa** , M. Costagli, C. Lapucci , M. Inglese

*Poster presentation at ECTRIMS 2022*

Deep multiple sclerosis lesion phenotyping using multimodal quantitative MRI

**Boffa G**, Schiavi S, Tazza F, Lapucci C, Piredda GF, Zacà D, Roccatagliata L, Hilbert T, Kober T, Inglese M, Costagli M

*Poster presentation at ISMRM 2023*

Hyperperfusion precedes demyelination in Multiple Sclerosis lesion formation : a multimodal MRI and [11C]-PiB PET study

Soulier T, Yazdan-Panah A, Hamzaoui M, **Boffa G**, Lazzarotto A, Ricigliano V.AG, Louapre C, Bodini B, Stankoff B

*Poster presentation at ISMRM 2023*

## BIBLIOGRAPHY


Absinta, M., Sati, P., Gaitán, M.I., Maggi, P., Cortese, I.C.M., Filippi, M., Reich, D.S., 2013. Seven-tesla phase imaging of acute multiple sclerosis lesions: A new window into the inflammatory process. Ann Neurol 74, 669–678. https://doi.org/10.1002/ana.23959

Absinta, M., Sati, P., Masuzzo, F., Nair, G., Sethi, V., Kolb, H., Ohayon, J., Wu, T., Cortese, I.C.M., Reich, D.S., 2019. Association of Chronic Active Multiple Sclerosis Lesions With Disability In Vivo. JAMA Neurol 76, 1474. https://doi.org/10.1001/jamaneurol.2019.2399

Absinta, M., Sati, P., Schindler, M., Leibovitch, E.C., Ohayon, J., Wu, T., Meani, A., Filippi, M., Jacobson, S., Cortese, I.C.M., Reich, D.S., 2016. Persistent 7-tesla phase rim predicts poor outcome in new multiple sclerosis patient lesions. Journal of Clinical Investigation 126, 2597–2609. https://doi.org/10.1172/JCI86198

Atkins, H.L., Bowman, M., Allan, D., Anstee, G., Arnold, D.L., Bar-Or, A., Bence-Bruckler, I., Birch, P., Bredeson, C., Chen, J., Fergusson, D., Halpenny, M., Hamelin, L., Huebsch, L., Hutton, B., Laneuville, P., Lapierre, Y., Lee, H., Martin, L., McDiarmid, S., O'Connor, P., Ramsay, T., Sabloff, M., Walker, L., Freedman, M.S., 2016. Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. The Lancet 388, 576–585. https://doi.org/10.1016/S0140-6736(16)30169-6

Bagnato, F., Hametner, S., Yao, B., van Gelderen, P., Merkle, H., Cantor, F.K., Lassmann, H., Duyn, J.H., 2011. Tracking iron in multiple sclerosis: A combined imaging and histopathological study at 7 Tesla. Brain 134, 3599–3612. https://doi.org/10.1093/brain/awr278

Barkhof, F., Kappos, L., Wolinsky, J.S., Li, D.K.B., Bar-Or, A., Hartung, H.P., Belachew, S., Han, J., Julian, L., Sauter, A., Napieralski, J., Koendgen, H., Hauser, S.L., 2019. Onset of clinical and MRI efficacy of ocrelizumab in relapsing multiple sclerosis. Neurology 93, e1778–e1786. https://doi.org/10.1212/WNL.0000000000008189

Blindenbacher, N., Brunner, E., Asseyer, S., Scheel, M., Siebert, N., Rasche, L., Bellmann-Strobl, J., Brandt, A., Ruprecht, K., Meier, D., Wuerfel, J., Paul, F., Sinnecker, T., 2020. Evaluation of the 'ring sign' and the 'core sign' as a magnetic resonance imaging marker of disease activity and progression in clinically isolated syndrome and early multiple sclerosis. Mult Scler J Exp Transl Clin 6, 205521732091548. https://doi.org/10.1177/2055217320915480

Bodini, B., Poirion, E., Tonietto, M., Benoit, C., Palladino, R., Maillart, E., Portera, E., Battaglini, M., Bera, G., Kuhnast, B., Louapre, C., Bottlaender, M., Stankoff, B., 2020. Individual mapping of innate immune cell activation is a candidate marker of patient-specific trajectories of worsening disability in multiple sclerosis. Journal of Nuclear Medicine 61, 1043. https://doi.org/10.2967/jnumed.119.231340

Bodini, B., Veronese, M., García-Lorenzo, D., Battaglini, M., Poirion, E., Chardain, A., Freeman, L., Louapre, C., Tchikviladze, M., Papeix, C., Dollé, F., Zalc, B., Lubetzki, C., Bottlaender, M., Turkheimer, F., Stankoff, B., 2016. Dynamic <scp>I</scp> maging of <scp>I</scp> ndividual <scp>R</scp> emyelination <scp>P</scp> rofiles in <scp>M</scp> ultiple <scp>S</scp> clerosis. Ann Neurol 79, 726–738. https://doi.org/10.1002/ana.24620

Boffa, G., Lapucci, C., Sbragia, E., Varaldo, R., Raiola, A.M., Currò, D., Roccatagliata, L., Capello, E., Laroni, A., Mikulska, M., Gualandi, F., Uccelli, A., Angelucci, E., Mancardi, G.L., Inglese, M., 2020. Aggressive multiple sclerosis: a single-centre, real-world treatment experience with autologous haematopoietic stem cell transplantation and alemtuzumab. Eur J Neurol 27, 2047–2055. https://doi.org/10.1111/ene.14324

Boffa, G., Massacesi, L., Inglese, M., Mariottini, A., Capobianco, M., Moiola, L., Amato, M.P., Cottone, S., Gualandi, F., de Gobbi, M., Greco, R., Scimè, R., Frau, J., Zimatore, G.B.,

Bertolotto, A., Comi, G., Uccelli, A., Signori, A., Angelucci, E., Innocenti, C., Ciceri, F., Repice, A.M., Sormani, M.P., Saccardi, R., Mancardi, G., 2021. Long-term Clinical Outcomes of Hematopoietic Stem Cell Transplantation in Multiple Sclerosis. Neurology 96, e1215–e1226. https://doi.org/10.1212/WNL.0000000000011461

Brittain, G., Coles, A.J., Giovannoni, G., Muraro, P.A., Palace, J., Petrie, J., Roldan, E., Scolding, N.J., Snowden, J.A., Sharrack, B., 2022. Autologous haematopoietic stem cell transplantation for immune-mediated neurological diseases: what, how, who and why? Pract Neurol pn-2022-003531. https://doi.org/10.1136/pn-2022-003531

Brown, R.A., Narayanan, S., Arnold, D.L., 2013. Segmentation of magnetization transfer ratio lesions for longitudinal analysis of demyelination and remyelination in multiple sclerosis. Neuroimage 66, 103–109. https://doi.org/10.1016/j.neuroimage.2012.10.059

Brownlee, W., Bourdette, D., Broadley, S., Killestein, J., Ciccarelli, O., 2020. Treating multiple sclerosis and neuromyelitis optica spectrum disorder during the COVID-19 pandemic. Neurology 94, 949–952. https://doi.org/10.1212/WNL.0000000000009507

Brownlee, W.J., Altmann, D.R., Prados, F., Miszkiel, K.A., Eshaghi, A., Gandini Wheeler-Kingshott, C.A.M., Barkhof, F., Ciccarelli, O., 2019a. Early imaging predictors of long-term outcomes in relapse-onset multiple sclerosis. Brain 142, 2276–2287. https://doi.org/10.1093/brain/awz156

Brownlee, W.J., Solanky, B., Prados, F., Yiannakas, M., da Mota, P., Riemer, F., Cardoso, M.J., Ourselin, S., Golay, X., Gandini Wheeler-Kingshott, C., Ciccarelli, O., 2019b. Cortical grey matter sodium accumulation is associated with disability and secondary progressive disease course in relapse-onset multiple sclerosis. J Neurol Neurosurg Psychiatry 90, 755–760. https://doi.org/10.1136/jnnp-2018-319634

Burman, J., Iacobaeus, E., Svenningsson, A., Lycke, J., Gunnarsson, M., Nilsson, P., Vrethem, M., Fredrikson, S., Martin, C., Sandstedt, A., Uggla, B., Lenhoff, S., Johansson, J.E., Isaksson, C., Hägglund, H., Carlson, K., Fagius, J., 2014. Autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: The Swedish experience. J Neurol Neurosurg Psychiatry 85, 1116–1121. https://doi.org/10.1136/jnnp-2013-307207

Burt, R.K., Balabanov, R., Burman, J., Sharrack, B., Snowden, J.A., Oliveira, M.C., Fagius, J., Rose, J., Nelson, F., Barreira, A.A., Carlson, K., Han, X., Moraes, D., Morgan, A., Quigley, K., Yaung, K., Buckley, R., Alldredge, C., Clendenan, A., Calvario, M.A., Henry, J., Jovanovic, B., Helenowski, I.B., 2019a. Effect of Nonmyeloablative Hematopoietic Stem Cell Transplantation vs Continued Disease-Modifying Therapy on Disease Progression in Patients With Relapsing-Remitting Multiple Sclerosis. JAMA 321, 165. https://doi.org/10.1001/jama.2018.18743

Burt, R.K., Balabanov, R., Burman, J., Sharrack, B., Snowden, J.A., Oliveira, M.C., Fagius, J., Rose, J., Nelson, F., Barreira, A.A., Carlson, K., Han, X., Moraes, D., Morgan, A., Quigley, K., Yaung, K., Buckley, R., Alldredge, C., Clendenan, A., Calvario, M.A., Henry, J., Jovanovic, B., Helenowski, I.B., 2019b. Effect of Nonmyeloablative Hematopoietic Stem Cell Transplantation vs Continued Disease-Modifying Therapy on Disease Progression in Patients With Relapsing-Remitting Multiple Sclerosis. JAMA 321, 165. https://doi.org/10.1001/jama.2018.18743

Burt, R.K., Balabanov, R., Han, X., Sharrack, B., Morgan, A., Quigley, K., Yaung, K., Helenowski, I.B., Jovanovic, B., Spahovic, D., Arnautovic, I., Lee, D.C., Benefield, B.C., Futterer, S., Oliveira, M.C., Burman, J., 2015. Association of Nonmyeloablative Hematopoietic Stem Cell Transplantation With Neurological Disability in Patients With Relapsing-Remitting Multiple Sclerosis. JAMA 313, 275. https://doi.org/10.1001/jama.2014.17986

Burt, R.K., Cohen, B.A., Russell, E., Spero, K., Joshi, A., Oyama, Y., Karpus, W.J., Luo, K., Jovanovic, B., Traynor, A., Karlin, K., Stefoski, D., Burns, W.H., 2003. Hematopoietic stem cell transplantation for progressive multiple sclerosis: failure of a total body irradiation-based

conditioning regimen to prevent disease progression in patients with high disability scores. Blood 102, 2373–8. https://doi.org/10.1182/blood-2003-03-0877

Burt, R.K., Han, X., Quigley, K., Helenowski, I.B., Balabanov, R., 2021a. Real-world application of autologous hematopoietic stem cell transplantation in 507 patients with multiple sclerosis. J Neurol. https://doi.org/10.1007/s00415-021-10820-2

Burt, R.K., Muraro, P.A., Farge, D., Oliveira, M.C., Snowden, J.A., Saccardi, R., Han, X., Quigley, K., Bueno, V., Frasca, D., Fedorenko, D., Burman, J., 2021b. New autoimmune diseases after autologous hematopoietic stem cell transplantation for multiple sclerosis. Bone Marrow Transplant 56, 1509–1517. https://doi.org/10.1038/s41409-021-01277-y

Capasso, N., Nozzolillo, A., Scalia, G., Lanzillo, R., Carotenuto, A., de Angelis, M., Petruzzo, M., Saccà, F., Russo, C.V., Brescia Morra, V., Moccia, M., 2021. Ocrelizumab depletes T-lymphocytes more than rituximab in multiple sclerosis. Mult Scler Relat Disord 49, 102802. https://doi.org/10.1016/j.msard.2021.102802

Casanova, B., Jarque, I., Gascón, F., Hernández-Boluda, J.C., Pérez-Miralles, F., de la Rubia, J., Alcalá, C., Sanz, J., Mallada, J., Cervelló, A., Navarré, A., Carcelén-Gadea, M., Boscá, I., Gil-Perotin, S., Solano, C., Sanz, M.A., Coret, F., 2017. Autologous hematopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: comparison with secondary progressive multiple sclerosis. Neurological Sciences 38, 1213–1221. https://doi.org/10.1007/s10072-017-2933-6

Cencioni, M.T., Genchi, A., Brittain, G., de Silva, T.I., Sharrack, B., Snowden, J.A., Alexander, T., Greco, R., Muraro, P.A., 2022. Immune Reconstitution Following Autologous Hematopoietic Stem Cell Transplantation for Multiple Sclerosis: A Review on Behalf of the EBMT Autoimmune Diseases Working Party. Front Immunol 12. https://doi.org/10.3389/fimmu.2021.813957

Chatterton, S., Withers, B., Sutton, I.J., Milliken, S.T., Ma, D.D., Moore, J.J., Massey, J.C., 2021. Pregnancy post autologous stem cell transplant with BEAM conditioning for multiple sclerosis. Multiple Sclerosis Journal 27, 2112–2115. https://doi.org/10.1177/13524585211005660

Chen, B., Zhou, M., Ouyang, J., Zhou, R., Xu, J., Zhang, Q., Yang, Y., Xu, Yong, Shao, X., Meng, L., Wang, J., Xu, Yun, Ni, X., Zhang, X., 2012. Long-term efficacy of autologous haematopoietic stem cell transplantation in multiple sclerosis at a single institution in China. Neurological Sciences 33, 881–886. https://doi.org/10.1007/s10072-011-0859-y

Chen, J.T., Collins, D.L., Atkins, H.L., Freedman, M.S., Arnold, D.L., 2008. Magnetization transfer ratio evolution with demyelination and remyelination in multiple sclerosis lesions. Ann Neurol 63, 254–262. https://doi.org/10.1002/ana.21302

Chen, J.T., Collins, D.L., Atkins, H.L., Freedman, M.S., Galal, A., Arnold, D.L., 2006. Brain atrophy after immunoablation and stem cell transplantation in multiple sclerosis. Neurology 66, 1935–1937. https://doi.org/10.1212/01.wnl.0000219816.44094.f8

Cohen, J.A., Baldassari, L.E., Atkins, H.L., Bowen, J.D., Bredeson, C., Carpenter, P.A., Corboy, J.R., Freedman, M.S., Grif, L.M., Lowsky, R., Majhail, N.S., Muraro, P.A., Nash, R.A., Pasquini, M.C., Sarantopoulos, S., Savani, B.N., Storek, J., Sullivan, K.M., Georges, G.E., 2019. Biology of Blood and Marrow Transplantation Autologous Hematopoietic Cell Transplantation for Treatment-Refractory Relapsing Multiple Sclerosis : Position Statement from the American Society for Blood and Marrow Transplantation 25. https://doi.org/10.1016/j.bbmt.2019.02.014

Cree, B.A.C., Gourraud, P., Oksenberg, J.R., Bevan, C., Crabtree-hartman, E., Gelfand, J.M., Goodin, D.S., Graves, J., Green, A.J., Mowry, E., Okuda, D.T., Pelletier, D., Zamvil, S.S., Agrawal, A., Caillier, S., Ciocca, C., Gomez, R., Kanner, R., Lincoln, R., Lizee, A., Qualley, P., Santaniello, A., Suleiman, L., Bucci, M., Panara, V., Papinutto, N., Stern, W.A., Zhu, A.H., Cutter, G.R.,

Baranzini, S., Henry, R.G., Hauser, S.L., 2016. Long-Term Evolution of Multiple Sclerosis Disability in the Treatment Era 499–510. https://doi.org/10.1002/ana.24747
Cree, B.A.C., Hollenbach, J.A., Bove, R., Kirkish, G., Sacco, S., Caverzasi, E., Bischof, A., Gundel, T., Zhu, A.H., Papinutto, N., Stern, W.A., Bevan, C., Romeo, A., Goodin, D.S., Gelfand, J.M., Graves, J., Green, A.J., Wilson, M.R., Zamvil, S.S., Zhao, C., Gomez, R., Ragan, N.R., Rush, G.Q., Barba, P., Santaniello, A., Baranzini, S.E., Oksenberg, J.R., Henry, R.G., Hauser, S.L., 2019. Silent progression in disease activity–free relapsing multiple sclerosis. Ann Neurol 85, 653–666. https://doi.org/10.1002/ana.25463
Curro, D., Vuolo, L., Gualandi, F., Bacigalupo, A., Roccatagliata, L., Capello, E., Uccelli, A., Saccardi, R., Sormani, M.P., Mancardi, G., 2015. Low intensity lympho-ablative regimen followed by autologous hematopoietic stem cell transplantation in severe forms of multiple sclerosis: A MRI-based clinical study. Multiple Sclerosis Journal 21, 1423–1430. https://doi.org/10.1177/1352458514564484
Daikeler, T., Labopin, M., di Gioia, M., Abinun, M., Alexander, T., Miniati, I., Gualandi, F., Fassas, A., Martin, T., Schwarze, C.P., Wulffraat, N., Buch, M., Sampol, A., Carreras, E., Dubois, B., Gruhn, B., Güngör, T., Pohlreich, D., Schuerwegh, A., Snarski, E., Snowden, J., Veys, P., Fasth, A., Lenhoff, S., Messina, C., Voswinkel, J., Badoglio, M., Henes, J., Launay, D., Tyndall, A., Gluckman, E., Farge, D., 2011. Secondary autoimmune diseases occurring after HSCT for an autoimmune disease: a retrospective study of the EBMT Autoimmune Disease Working Party. Blood 118, 1693–1698. https://doi.org/10.1182/blood-2011-02-336156
Dal-Bianco, A., Grabner, G., Kronnerwetter, C., Weber, M., Höftberger, R., Berger, T., Auff, E., Leutmezer, F., Trattnig, S., Lassmann, H., Bagnato, F., Hametner, S., 2017. Slow expansion of multiple sclerosis iron rim lesions: pathology and 7 T magnetic resonance imaging. Acta Neuropathol 133, 25–42. https://doi.org/10.1007/s00401-016-1636-z
Dal-Bianco, A., Grabner, G., Kronnerwetter, C., Weber, M., Kornek, B., Kasprian, G., Berger, T., Leutmezer, F., Rommer, P.S., Trattnig, S., Lassmann, H., Hametner, S., 2021. Long-term evolution of multiple sclerosis iron rim lesions in 7 T MRI. Brain. https://doi.org/10.1093/brain/awaa436
Das, J., Snowden, J., Burman, J., Freedman, M., Atkins, H., Bowman, M., Burt, R., Saccardi, R., Innocenti, C., Mistry, S., Laud, P., Jessop, H., Sharrack, B., 2021. Autologous haematopoietic stem cell transplantation as a first-line disease-modifying therapy in patients with 'aggressive' multiple sclerosis. Multiple Sclerosis Journal 27, 1198–1204. https://doi.org/10.1177/1352458520985238
de Stefano, N., Arnold, D.L., 2015. Towards a better understanding of *pseudoatrophy* in the brain of multiple sclerosis patients. Multiple Sclerosis Journal 21, 675–676. https://doi.org/10.1177/1352458514564494
Deistung, A., Schäfer, A., Schweser, F., Biedermann, U., Turner, R., Reichenbach, J.R., 2013. Toward in vivo histology: A comparison of quantitative susceptibility mapping (QSM) with magnitude-, phase-, and R2*-imaging at ultra-high magnetic field strength. Neuroimage 65, 299–314. https://doi.org/10.1016/j.neuroimage.2012.09.055
Delforge, J., Spelle, L., Bendriem, B., Samson, Y., Syrota, A., 1997. Parametric Images of Benzodiazepine Receptor Concentration Using a Partial-Saturation Injection. Journal of Cerebral Blood Flow & Metabolism 17, 343–355. https://doi.org/10.1097/00004647-199703000-00011
Dubinsky, A.N., Burt, R.K., Martin, R., Muraro, P.A., 2010. T-cell clones persisting in the circulation after autologous hematopoietic SCT are undetectable in the peripheral CD34+ selected graft. Bone Marrow Transplant 45, 325–331. https://doi.org/10.1038/bmt.2009.139

ECTRIMS 2021 – Late Breaking News Oral Presentations, 2021. . Multiple Sclerosis Journal 27, 741–751. https://doi.org/10.1177/13524585211047081
Edan, G., Comi, G., le Page, E., Leray, E., Rocca, M.A., Filippi, M., 2011. Mitoxantrone prior to interferon beta-1b in aggressive relapsing multiple sclerosis: a 3-year randomised trial. J Neurol Neurosurg Psychiatry 82, 1344–1350. https://doi.org/10.1136/jnnp.2010.229724
Eisele, P., Wittayer, M., Weber, C.E., Platten, M., Schirmer, L., Gass, A., 2022. Impact of disease-modifying therapies on evolving tissue damage in iron rim multiple sclerosis lesions. Multiple Sclerosis Journal 28, 2294–2298. https://doi.org/10.1177/13524585221106338
Fassas, A., Kimiskidis, V.K., Sakellari, I., Kapinas, K., Anagnostopoulos, A., Tsimourtou, V., Sotirakoglou, K., Kazis, A., 2011. Long-term results of stem cell transplantation for MS: A single-center experience. Neurology 76, 1066–1070. https://doi.org/10.1212/WNL.0b013e318211c537
Fernández-Velasco, J.I., Kuhle, J., Monreal, E., Meca-Lallana, V., Meca-Lallana, J., Izquierdo, G., Gascón-Giménez, F., Sainz de la Maza, S., Walo-Delgado, P.E., Maceski, A., Rodríguez-Martín, E., Roldán, E., Villarrubia, N., Saiz, A., Blanco, Y., Sánchez, P., Carreón-Guarnizo, E., Aladro, Y., Brieva, L., Íñiguez, C., González-Suárez, I., Rodríguez de Antonio, L.A., Masjuan, J., Costa-Frossard, L., Villar, L.M., 2021. Effect of Ocrelizumab in Blood Leukocytes of Patients With Primary Progressive MS. Neurology - Neuroimmunology Neuroinflammation 8, e940. https://doi.org/10.1212/NXI.0000000000000940
Frau, J., Carai, M., Coghe, G., Fenu, G., Lorefice, L., la Nasa, G., Mamusa, E., Vacca, A., Marrosu, M.G., Cocco, E., 2018. Long-term follow-up more than 10 years after HSCT: a monocentric experience. J Neurol 265, 410–416. https://doi.org/10.1007/s00415-017-8718-2
Freeman, L., Garcia-Lorenzo, D., Bottin, L., Leroy, C., Louapre, C., Bodini, B., Papeix, C., Assouad, R., Granger, B., Tourbah, A., Dollé, F., Lubetzki, C., Bottlaender, M., Stankoff, B., 2015. The neuronal component of gray matter damage in multiple sclerosis: A [11C]flumazenil positron emission tomography study. Ann Neurol 78, 554–567. https://doi.org/10.1002/ana.24468
Frischer, J.M., Weigand, S.D., Guo, Y., Kale, N., Parisi, J.E., Pirko, I., Mandrekar, J., Bramow, S., Metz, I., Brück, W., Lassmann, H., Lucchinetti, C.F., 2015. Clinical and pathological insights into the dynamic nature of the white matter multiple sclerosis plaque. Ann Neurol 78, 710–721. https://doi.org/10.1002/ana.24497
Geurts, J.J.G., Roosendaal, S.D., Calabrese, M., Ciccarelli, O., Agosta, F., Chard, D.T., Gass, A., Huerga, E., Moraal, B., Pareto, D., Rocca, M.A., Wattjes, M.P., Yousry, T.A., Uitdehaag, B.M.J., Barkhof, F., 2011. Consensus recommendations for MS cortical lesion scoring using double inversion recovery MRI. Neurology 76, 418–424. https://doi.org/10.1212/WNL.0b013e31820a0cc4
Gholipour, T., Healy, B., Baruch, N.F., Weiner, H.L., Chitnis, T., 2011. Demographic and clinical characteristics of malignant multiple sclerosis. Neurology 76, 1996–2001. https://doi.org/10.1212/WNL.0b013e31821e559d
Gillen, K.M., Mubarak, M., Park, C., Ponath, G., Zhang, S., Dimov, A., Levine-Ritterman, M., Toro, S., Huang, W., Amici, S., Kaunzner, U.W., Gauthier, S.A., Guerau-de-Arellano, M., Wang, Y., Nguyen, T.D., Pitt, D., 2021. QSM is an imaging biomarker for chronic glial activation in multiple sclerosis lesions. Ann Clin Transl Neurol 8, 877–886. https://doi.org/10.1002/acn3.51338
Gingele, S., Jacobus, T., Konen, F., Hümmert, M., Sühs, K.-W., Schwenkenbecher, P., Ahlbrecht, J., Möhn, N., Müschen, L., Bönig, L., Alvermann, S., Schmidt, R., Stangel, M., Jacobs, R., Skripuletz, T., 2018. Ocrelizumab Depletes CD20+ T Cells in Multiple Sclerosis Patients. Cells 8, 12. https://doi.org/10.3390/cells8010012

Giovannoni, G., Popescu, V., Wuerfel, J., Hellwig, K., Iacobeus, E., Jensen, M.B., García-Domínguez, J.M., Sousa, L., de Rossi, N., Hupperts, R., Fenu, G., Bodini, B., Kuusisto, H.-M., Stankoff, B., Lycke, J., Airas, L., Granziera, C., Scalfari, A., 2022. Smouldering multiple sclerosis: the 'real MS.' Ther Adv Neurol Disord 15, 175628642110667. https://doi.org/10.1177/17562864211066751

Hametner, S., Wimmer, I., Haider, L., Pfeifenbring, S., Brück, W., Lassmann, H., 2013. Iron and neurodegeneration in the multiple sclerosis brain. Ann Neurol 74, 848–861. https://doi.org/10.1002/ana.23974

Hammond, K.E., Metcalf, M., Carvajal, L., Okuda, D.T., Srinivasan, R., Vigneron, D., Nelson, S.J., Pelletier, D., 2008. Quantitative in vivo magnetic resonance imaging of multiple sclerosis at 7 Tesla with sensitivity to iron. Ann Neurol 64, 707–713. https://doi.org/10.1002/ana.21582

Harris, K.M., Lim, N., Lindau, P., Robins, H., Griffith, L.M., Nash, R.A., Turka, L.A., Muraro, P.A., 2020. Extensive intrathecal T cell renewal following hematopoietic transplantation for multiple sclerosis. JCI Insight 5. https://doi.org/10.1172/jci.insight.127655

Harris, K.M., Lu, T., Lim, N., Turka, L.A., 2018. Challenges and opportunities for biomarkers of clinical response to AHSCT in autoimmunity. Front Immunol 9, 1–9. https://doi.org/10.3389/fimmu.2018.00100

Harrison, D.M., Wang, K.Y., Fiol, J., Naunton, K., Royal, W., Hua, J., Izbudak, I., 2017. Leptomeningeal Enhancement at 7T in Multiple Sclerosis: Frequency, Morphology, and Relationship to Cortical Volume. Journal of Neuroimaging 27, 461–468. https://doi.org/10.1111/jon.12444

Hauser, S.L., Kappos, L., Arnold, D.L., Bar-Or, A., Brochet, B., Naismith, R.T., Traboulsee, A., Wolinsky, J.S., Belachew, S., Koendgen, H., Levesque, V., Manfrini, M., Model, F., Hubeaux, S., Mehta, L., Montalban, X., 2020. Five years of ocrelizumab in relapsing multiple sclerosis: OPERA studies open-label extension. Neurology 95, e1854–e1867. https://doi.org/10.1212/WNL.0000000000010376

Häußler, V., Ufer, F., Pöttgen, J., Wolschke, C., Friese, M.A., Kröger, N., Heesen, C., Stellmann, J., 2021. aHSCT is superior to alemtuzumab in maintaining NEDA and improving cognition in multiple sclerosis. Ann Clin Transl Neurol 8, 1269–1278. https://doi.org/10.1002/acn3.51366

Havrdova, E., Horakova, D., Kovarova, I., 2015. Alemtuzumab in the treatment of multiple sclerosis : key clinical trial results and considerations for use 31–45. https://doi.org/10.1177/1756285614563522

He, A., Merkel, B., Brown, J.W.L., Zhovits Ryerson, L., Kister, I., Malpas, C.B., Sharmin, S., Horakova, D., Kubala Havrdova, E., Spelman, T., Izquierdo, G., Eichau, S., Trojano, M., Lugaresi, A., Hupperts, R., Sola, P., Ferraro, D., Lycke, J., Grand'Maison, F., Prat, A., Girard, M., Duquette, P., Larochelle, C., Svenningsson, A., Petersen, T., Grammond, P., Granella, F., van Pesch, V., Bergamaschi, R., McGuigan, C., Coles, A., Hillert, J., Piehl, F., Butzkueven, H., Kalincik, T., 2020. Timing of high-efficacy therapy for multiple sclerosis: a retrospective observational cohort study. Lancet Neurol 19, 307–316. https://doi.org/10.1016/S1474-4422(20)30067-3

Hemond, C.C., Baek, J., Ionete, C., Reich, D.S., 2022. Paramagnetic rim lesions are associated with pathogenic CSF profiles and worse clinical status in multiple sclerosis: A retrospective cross-sectional study. Multiple Sclerosis Journal 28, 2046–2056. https://doi.org/10.1177/13524585221102921

Huiskamp, M., Kiljan, S., Kulik, S., Witte, M.E., Jonkman, L.E., GJM Bol, J., Schenk, G.J., Hulst, H.E., Tewarie, P., Schoonheim, M.M., Geurts, J.J., 2022. Inhibitory synaptic loss drives network changes in multiple sclerosis: An ex vivo to in silico translational study. Multiple Sclerosis Journal 28, 2010–2019. https://doi.org/10.1177/13524585221125381

Iacobaeus, E., Arrambide, G., Pia Amato, M., Derfuss, T., Vukusic, S., Hemmer, B., Tintore, M., Brundin, L., 2020. Aggressive multiple sclerosis (1): Towards a definition of the phenotype. Multiple Sclerosis Journal 1–14. https://doi.org/10.1177/1352458520925369

Kang, Y., Rúa, S.M.H., Kaunzner, U.W., Perumal, J., Nealon, N., Qu, W., Kothari, P.J., Vartanian, T., Kuceyeski, A., Gauthier, S.A., 2020. A Multi-Ligand Imaging Study Exploring GABAergic Receptor Expression and Inflammation in Multiple Sclerosis. Mol Imaging Biol 22, 1600–1608. https://doi.org/10.1007/s11307-020-01501-z

Kappos, L., Wolinsky, J.S., Giovannoni, G., Arnold, D.L., Wang, Q., Bernasconi, C., Model, F., Koendgen, H., Manfrini, M., Belachew, S., Hauser, S.L., 2020. Contribution of Relapse-Independent Progression vs Relapse-Associated Worsening to Overall Confirmed Disability Accumulation in Typical Relapsing Multiple Sclerosis in a Pooled Analysis of 2 Randomized Clinical Trials. JAMA Neurol 77, 1132. https://doi.org/10.1001/jamaneurol.2020.1568

Kolb, H., Absinta, M., Beck, E.S., Ha, S., Song, Y., Norato, G., Cortese, I., Sati, P., Nair, G., Reich, D.S., 2021. <scp>7T MRI</scp> Differentiates Remyelinated from Demyelinated Multiple Sclerosis Lesions. Ann Neurol 90, 612–626. https://doi.org/10.1002/ana.26194

Krasulová, E., Trněný, M., Kozák, T., Vacková, B., Pohlreich, D., Kemlink, D., Kobylka, P., Kovářová, I., Lhotáková, P., Havrdová, E., 2010. High-dose immunoablation with autologous haematopoietic stem cell transplantation in aggressive multiple sclerosis: a single centre 10-year experience. Multiple Sclerosis Journal 16, 685–693. https://doi.org/10.1177/1352458510364538

Kuhlmann, T., Ludwin, S., Prat, A., Antel, J., Brück, W., Lassmann, H., 2017. An updated histological classification system for multiple sclerosis lesions. Acta Neuropathol 133, 13–24. https://doi.org/10.1007/s00401-016-1653-y

Kuhlmann, T., Moccia, M., Coetzee, T., Cohen, J.A., Correale, J., Graves, J., Marrie, R.A., Montalban, X., Yong, V.W., Thompson, A.J., Reich, D.S., Amato, M.P., Banwell, B., Barkhof, F., Chataway, J., Chitnis, T., Comi, G., Derfuss, T., Finlayson, M., Goldman, M., Green, A., Hellwig, K., Kos, D., Miller, A., Mowry, E., Oh, J., Salter, A., Sormani, , Maria Pia, Tintore, M., Tremlett, , Helen, Trojano, M., van der Walt, A., Vukusic, S., Waubant, E., 2023. Multiple sclerosis progression: time for a new mechanism-driven framework. Lancet Neurol 22, 78–88. https://doi.org/10.1016/S1474-4422(22)00289-7

Kvistad, S.A.S., Lehmann, A.K., Trovik, L.H., Kristoffersen, E.K., Bø, L., Myhr, K.-M., Torkildsen, Ø., 2019. Safety and efficacy of autologous hematopoietic stem cell transplantation for multiple sclerosis in Norway. Multiple Sclerosis Journal 135245851989392. https://doi.org/10.1177/1352458519893926

Kyrcz-Krzemień, S., Helbig, G., Torba, K., Koclęga, A., Krawczyk-Kuliś, M., 2016. Safety and efficacy of hematopoietic stem cells mobilization in patients with multiple sclerosis. Hematology 21, 42–45. https://doi.org/10.1179/1607845415Y.0000000049

Lazzarotto, A., Tonietto, M., Poirion, E., Battaglini, M., Palladino, R., Benoit, C., Ricigliano, V.A., Maillart, E., de Stefano, N., Stankoff, B., Bodini, B., 2022. Clinically relevant profiles of myelin content changes in patients with multiple sclerosis: A multimodal and multicompartment imaging study. Multiple Sclerosis Journal 28, 1881–1890. https://doi.org/10.1177/13524585221096975

Lee, H., Nakamura, K., Narayanan, S., Brown, R., Chen, J., Atkins, H.L., Freedman, M.S., Arnold, D.L., 2018. Impact of immunoablation and autologous hematopoietic stem cell transplantation on gray and white matter atrophy in multiple sclerosis. Multiple Sclerosis Journal 24, 1055–1066. https://doi.org/10.1177/1352458517715811

Lee, H., Nakamura, K., Narayanan, S., Brown, R.A., Nash, R.A., Griffith, L.M., Steinmiller, K.C., Devine, S.M., Hutton, G.J., Popat, U., Racke, M.K., Georges, G.E., Bowen, J.D., Arnold, D.L.,

2021. Brain volume change after high-dose immunosuppression and autologous hematopoietic cell transplantation for relapsing-remitting multiple sclerosis. Mult Scler Relat Disord 54, 103149. https://doi.org/10.1016/j.msard.2021.103149
Lee, H., Narayanan, S., Brown, R.A., Chen, J.T., Atkins, H.L., Freedman, M.S., Arnold, D.L., 2017a. Brain atrophy after bone marrow transplantation for treatment of multiple sclerosis. Multiple Sclerosis Journal 23, 420–431. https://doi.org/10.1177/1352458516650992
Lee, H., Narayanan, S., Brown, R.A., Chen, J.T., Atkins, H.L., Freedman, M.S., Arnold, D.L., 2017b. Brain atrophy after bone marrow transplantation for treatment of multiple sclerosis. Multiple Sclerosis Journal 23, 420–431. https://doi.org/10.1177/1352458516650992
Lee, H., Narayanan, S., Brown, R.A., Chen, J.T., Atkins, H.L., Freedman, M.S., Arnold, D.L., 2016. Brain atrophy after bone marrow transplantation for treatment of multiple sclerosis 1–12. https://doi.org/10.1177/1352458516650992
Lehto, J., Sucksdorff, M., Nylund, M., Raitanen, R., Matilainen, M., Airas, L., 2022. PET-measurable innate immune cell activation reduction in chronic active lesions in PPMS brain after rituximab treatment: a case report. J Neurol. https://doi.org/10.1007/s00415-022-11539-4
Lloyd, A.F., Miron, V.E., 2019. The pro-remyelination properties of microglia in the central nervous system. Nat Rev Neurol 15, 447–458. https://doi.org/10.1038/s41582-019-0184-2
Lorscheider, J., Buzzard, K., Jokubaitis, V., Spelman, T., Havrdova, E., Horakova, D., Trojano, M., Izquierdo, G., Girard, M., Duquette, P., Prat, A., Lugaresi, A., Grand'maison, F., Grammond, P., Hupperts, R., Alroughani, R., Sola, P., Boz, C., Pucci, E., Lechner-Scott, J., Bergamaschi, R., Oreja-Guevara, C., Iuliano, G., van Pesch, V., Granella, F., Ramo-Tello, C., Spitaleri, D., Petersen, T., Slee, M., Verheul, F., Ampapa, R., Amato, M.P., Mccombe, P., Vucic, S., Sánchez Menoyo, J.L., Cristiano, E., Barnett, M.H., Hodgkinson, S., Olascoaga, J., Saladino, M.L., Gray, O., Shaw, C., Moore, F., Butzkueven, H., Kalincik, T., 2016. Defining secondary progressive multiple sclerosis. Brain 139, 2395–2405. https://doi.org/10.1093/brain/aww173
Lublin, F.D., Coetzee, T., Cohen, J.A., Marrie, R.A., Thompson, A.J., 2020. The 2013 clinical course descriptors for multiple sclerosis. Neurology 94, 1088–1092. https://doi.org/10.1212/WNL.0000000000009636
Lublin, F.D., Reingold, S.C., 1996. Defining the clinical course of multiple sclerosis: Results of an international survey. Neurology 46, 907–911. https://doi.org/10.1212/WNL.46.4.907
Lublin, F.D., Reingold, S.C., Cohen, J.A., Lublin, F.D., Reingold, S.C., Cohen, J.A., Cutter, G.R., Thompson, A.J., Wolinsky, J.S., Fox, R.J., Freedman, M.S., Goodman, A.D., Lubetzki, C., 2014. Defining the clinical course of multiple sclerosis : The 2013 revisions Defining the clinical course of multiple sclerosis The 2013 revisions. https://doi.org/10.1212/WNL.0000000000000560
Maggi, P., Kuhle, J., Schädelin, S., van der Meer, F., Weigel, M., Galbusera, R., Mathias, A., Lu, P.-J., Rahmanzadeh, R., Benkert, P., la Rosa, F., Bach Cuadra, M., Sati, P., Théaudin, M., Pot, C., van Pesch, V., Leppert, D., Stadelmann, C., Kappos, L., du Pasquier, R., Reich, D.S., Absinta, M., Granziera, C., 2021. Chronic White Matter Inflammation and Serum Neurofilament Levels in Multiple Sclerosis. Neurology 97, e543–e553. https://doi.org/10.1212/WNL.0000000000012326
Maggi, P., Sati, P., Nair, G., Cortese, I.C.M., Jacobson, S., Smith, B.R., Nath, A., Ohayon, J., van Pesch, V., Perrotta, G., Pot, C., Théaudin, M., Martinelli, V., Scotti, R., Wu, T., du Pasquier, R., Calabresi, P.A., Filippi, M., Reich, D.S., Absinta, M., 2020. Paramagnetic rim lesions are specific to multiple sclerosis: an international multicenter 3T MRI study. Ann Neurol ana.25877. https://doi.org/10.1002/ana.25877
Magliozzi, R., Fadda, G., Brown, R.A., Bar-Or, A., Howell, O.W., Hametner, S., Marastoni, D., Poli, A., Nicholas, R., Calabrese, M., Monaco, S., Reynolds, R., 2022. "Ependymal-in" Gradient of

Thalamic Damage in Progressive Multiple Sclerosis. Ann Neurol 92, 670–685. https://doi.org/10.1002/ana.26448
Magliozzi, R., Howell, O.W., Nicholas, R., Cruciani, C., Castellaro, M., Romualdi, C., Rossi, S., Pitteri, M., Benedetti, M.D., Gajofatto, A., Pizzini, F.B., Montemezzi, S., Rasia, S., Capra, R., Bertoldo, A., Facchiano, F., Monaco, S., Reynolds, R., Calabrese, M., 2018. Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. Ann Neurol 83, 739–755. https://doi.org/10.1002/ana.25197
Mainero, C., Louapre, C., Govindarajan, S.T., Gianni, C., Scott Nielsen, A., Cohen-Adad, J., Sloane, J., Kinkel, R.P., 2015. A gradient in cortical pathology in multiple sclerosis by in vivo quantitative 7 T imaging. Brain 138, 932–945. https://doi.org/10.1093/brain/awv011
Malpas, C.B., Manouchehrinia, A., Sharmin, S., Roos, I., Horakova, D., Havrdova, E.K., Trojano, M., Izquierdo, G., Eichau, S., Bergamaschi, R., Sola, P., Ferraro, D., Lugaresi, A., Prat, A., Girard, M., Duquette, P., Grammond, P., Grand'Maison, F., Ozakbas, S., van Pesch, V., Granella, F., Hupperts, R., Pucci, E., Boz, C., Sidhom, Y., Gouider, R., Spitaleri, D., Soysal, A., Petersen, T., Verheul, F., Karabudak, R., Turkoglu, R., Ramo-Tello, C., Terzi, M., Cristiano, E., Slee, M., McCombe, P., Macdonell, R., Fragoso, Y., Olascoaga, J., Altintas, A., Olsson, T., Butzkueven, H., Hillert, J., Kalincik, T., 2020. Early clinical markers of aggressive multiple sclerosis. Brain 143, 1400–1413. https://doi.org/10.1093/brain/awaa081
Mancardi, G., Sormani, M.P., Muraro, P.A., Boffa, G., Saccardi, R., 2018. Intense immunosuppression followed by autologous haematopoietic stem cell transplantation as a therapeutic strategy in aggressive forms of multiple sclerosis. Multiple Sclerosis Journal 24, 245–255. https://doi.org/10.1177/1352458517742532
Mancardi, G.L., Saccardi, R., Filippi, M., Gualandi, F., Murialdo, A., Inglese, M., Marrosu, M.G., Meucci, G., Massacesi, L., Lugaresi, A., Pagliai, F., Sormani, M.P., Sardanelli, F., Marmont, A., 2001. Autologous hematopoietic stem cell transplantation suppresses Gd-enhanced MRI activity in MS. Neurology 57, 62–68. https://doi.org/10.1212/WNL.57.1.62
Mancardi, G.L., Sormani, M.P., Gualandi, F., Saiz, A., Carreras, E., Merelli, E., Donelli, A., Lugaresi, A., di Bartolomeo, P., Rottoli, M.R., Rambaldi, A., Amato, M.P., Massacesi, L., di Gioia, M., Vuolo, L., Curro, D., Roccatagliata, L., Filippi, M., Aguglia, U., Iacopino, P., Farge, D., Saccardi, R., 2015. Autologous hematopoietic stem cell transplantation in multiple sclerosis: A phase II trial. Neurology 84, 981–988. https://doi.org/10.1212/WNL.0000000000001329
Mariottini, A., Bulgarini, G., Forci, B., Innocenti, C., Mealli, F., Mattei, A., Ceccarelli, C., Repice, A.M., Barilaro, A., Mechi, C., Saccardi, R., Massacesi, L., 2022. Autologous haematopoietic stem cell transplantation versus low-dose immunosuppression in secondary–progressive multiple sclerosis. Eur J Neurol 29, 1708–1718. https://doi.org/10.1111/ene.15280
Mariottini, A., Filippini, S., Innocenti, C., Forci, B., Mechi, C., Barilaro, A., Fani, A., Carlucci, G., Saccardi, R., Massacesi, L., Repice, A.M., 2021. Impact of autologous haematopoietic stem cell transplantation on disability and brain atrophy in secondary progressive multiple sclerosis. Multiple Sclerosis Journal 27, 61–70. https://doi.org/10.1177/1352458520902392
Massarotti, C., Sbragia, E., Boffa, G., Vercelli, C., Zimatore, G.B., Cottone, S., Frau, J., Raiola, A., Varaldo, R., Mancardi, G., Inglese, M., Anserini, P., 2021. Menstrual cycle resumption and female fertility after autologous hematopoietic stem cell transplantation for multiple sclerosis. Multiple Sclerosis Journal 27, 2103–2107. https://doi.org/10.1177/13524585211000616
McGinley, M.P., Goldschmidt, C.H., Rae-Grant, A.D., 2021. Diagnosis and Treatment of Multiple Sclerosis. JAMA 325, 765. https://doi.org/10.1001/jama.2020.26858
Mehra, V., Rhone, E., Widya, S., Zuckerman, M., Potter, V., Raj, K., Kulasekararaj, A., McLornan, D., de Lavallade, H., Benson-Quarm, N., Lim, C., Ware, S., Sudhanva, M., Malik, O., Nicholas, R.,

Muraro, P.A., Marsh, J., Mufti, G.J., Silber, E., Pagliuca, A., Kazmi, M.A., 2019. Epstein-Barr Virus and Monoclonal Gammopathy of Clinical Significance in Autologous Stem Cell Transplantation for Multiple Sclerosis. Clinical Infectious Diseases 69, 1757–1763. https://doi.org/10.1093/cid/ciz047

Menon, S., Zhu, F., Shirani, A., Oger, J., Freedman, M.S., Tremlett, H., 2017. Disability progression in aggressive multiple sclerosis. Multiple Sclerosis Journal 23, 456–463. https://doi.org/10.1177/1352458516653273

Metz, I., Lucchinetti, C.F., Openshaw, H., Garcia-Merino, A., Lassmann, H., Freedman, M.S., Atkins, H.L., Azzarelli, B., Kolar, O.J., Bruck, W., 2007. Autologous haematopoietic stem cell transplantation fails to stop demyelination and neurodegeneration in multiple sclerosis. Brain 130, 1254–1262. https://doi.org/10.1093/brain/awl370

Montalban, X., Gold, R., Thompson, A.J., Otero-Romero, S., Amato, M.P., Chandraratna, D., Clanet, M., Comi, G., Derfuss, T., Fazekas, F., Hartung, H.P., Havrdova, E., Hemmer, B., Kappos, L., Liblau, R., Lubetzki, C., Marcus, E., Miller, D.H., Olsson, T., Pilling, S., Selmaj, K., Siva, A., Sorensen, P.S., Sormani, M.P., Thalheim, C., Wiendl, H., Zipp, F., 2018. ECTRIMS/EAN Guideline on the pharmacological treatment of people with multiple sclerosis. Multiple Sclerosis Journal 24, 96–120. https://doi.org/10.1177/1352458517751049

Moore, J.J., Massey, J.C., Ford, C.D., Khoo, M.L., Zaunders, J.J., Hendrawan, K., Barnett, Y., Barnett, M.H., Kyle, K.A., Zivadinov, R., Ma, K.C., Milliken, S.T., Sutton, I.J., Ma, D.D.F., 2019. Prospective phase II clinical trial of autologous haematopoietic stem cell transplant for treatment refractory multiple sclerosis. J Neurol Neurosurg Psychiatry 90, 514–521. https://doi.org/10.1136/jnnp-2018-319446

Mumtaz, I.M., Hoyer, B.F., Panne, D., Moser, K., Winter, O., Cheng, Q.Y., Yoshida, T., Burmester, G.-R., Radbruch, A., Manz, R.A., Hiepe, F., 2012. Bone marrow of NZB/W mice is the major site for plasma cells resistant to dexamethasone and cyclophosphamide: Implications for the treatment of autoimmunity. J Autoimmun 39, 180–188. https://doi.org/10.1016/j.jaut.2012.05.010

Muraro, P.A., Pasquini, M., Atkins, H.L., Bowen, J.D., Farge, D., Fassas, A., Freedman, M.S., Georges, G.E., Gualandi, F., Hamerschlak, N., Havrdova, E., Kimiskidis, V.K., Kozak, T., Mancardi, G.L., Massacesi, L., Moraes, D.A., Nash, R.A., Pavletic, S., Ouyang, J., Rovira, M., Saiz, A., Simoes, B., Trnený, M., Zhu, L., Badoglio, M., Zhong, X., Sormani, M.P., Saccardi, R., 2017. Long-term Outcomes After Autologous Hematopoietic Stem Cell Transplantation for Multiple Sclerosis. JAMA Neurol 74, 459. https://doi.org/10.1001/jamaneurol.2016.5867

Nabizadeh, F., Pirahesh, K., Rafiei, N., Afrashteh, F., Ahmadabad, M.A., Zabeti, A., Mirmosayyeb, O., 2022. Autologous Hematopoietic Stem-Cell Transplantation in Multiple Sclerosis: A Systematic Review and Meta-Analysis. Neurol Ther 11, 1553–1569. https://doi.org/10.1007/s40120-022-00389-x

Nash, R.A., Dansey, R., Storek, J., Georges, G.E., Bowen, J.D., Holmberg, L.A., Kraft, G.H., Mayes, M.D., McDonagh, K.T., Chen, C.-S., DiPersio, J., LeMaistre, C.F., Pavletic, S., Sullivan, K.M., Sunderhaus, J., Furst, D.E., McSweeney, P.A., 2003. Epstein-Barr virus-associated posttransplantation lymphoproliferative disorder after high-dose immunosuppressive therapy and autologous CD34-selected hematopoietic stem cell transplantation for severe autoimmune diseases. Biology of Blood and Marrow Transplantation 9, 583–591. https://doi.org/10.1016/S1083-8791(03)00228-3

Nash, R.A., Hutton, G.J., Racke, M.K., Popat, U., Devine, S.M., Steinmiller, K.C., Griffith, L.M., Muraro, P.A., Openshaw, H., Sayre, P.H., Stuve, O., Arnold, D.L., Wener, M.H., Georges, G.E., Wundes, A., Kraft, G.H., Bowen, J.D., 2017. High-dose immunosuppressive therapy and

autologous HCT for relapsing-remitting MS. Neurology 88, 842–852. https://doi.org/10.1212/WNL.0000000000003660
Nicholas, R.S., Rhone, E.E., Mariottini, A., Silber, E., Malik, O., Singh-Curry, V., Turner, B., Scalfari, A., Ciccarelli, O., Sormani, M.P., Olavarria, E., Mehra, V., Gabriel, I., Kazmi, M.A., Muraro, P., 2021. Autologous Hematopoietic Stem Cell Transplantation in Active Multiple Sclerosis. Neurology 97, e890–e901. https://doi.org/10.1212/WNL.0000000000012449
Openshaw, H., Stuve, O., Antel, J.P., Nash, R., Lund, B.T., Weiner, L.P., Kashyap, A., McSweeney, P., Forman, S., 2000. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. Neurology 54, 2147–2150. https://doi.org/10.1212/WNL.54.11.2147
Parks, N.E., Flanagan, E.P., Lucchinetti, C.F., Wingerchuk, D.M., 2017. NEDA treatment target? No evident disease activity as an actionable outcome in practice. J Neurol Sci 383, 31–34. https://doi.org/10.1016/j.jns.2017.10.015
Pérez-Miralles, F., Sastre-Garriga, J., Tintoré, M., Arrambide, G., Nos, C., Perkal, H., Río, J., Edo, M., Horga, A., Castilló, J., Auger, C., Huerga, E., Rovira, A., Montalban, X., 2013. Clinical impact of early brain atrophy in clinically isolated syndromes. Multiple Sclerosis Journal 19, 1878–1886. https://doi.org/10.1177/1352458513488231
Poirion, E., Tonietto, M., Lejeune, F.X., Ricigliano, V.A.G., Boudot de la Motte, M., Benoit, C., Bera, G., Kuhnast, B., Bottlaender, M., Bodini, B., Stankoff, B., 2021. Structural and Clinical Correlates of a Periventricular Gradient of Neuroinflammation in Multiple Sclerosis. Neurology 96, e1865–e1875. https://doi.org/10.1212/WNL.0000000000011700
Popescu, B.F., Frischer, J.M., Webb, S.M., Tham, M., Adiele, R.C., Robinson, C.A., Fitz-Gibbon, P.D., Weigand, S.D., Metz, I., Nehzati, S., George, G.N., Pickering, I.J., Brück, W., Hametner, S., Lassmann, H., Parisi, J.E., Yong, G., Lucchinetti, C.F., 2017. Pathogenic implications of distinct patterns of iron and zinc in chronic MS lesions. Acta Neuropathol 134, 45–64. https://doi.org/10.1007/s00401-017-1696-8
Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L., Petersen, S.E., 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. Neuroimage 59, 2142–2154. https://doi.org/10.1016/j.neuroimage.2011.10.018
Preziosa, P., Kiljan, S., Steenwijk, M.D., Meani, A., van de Berg, W.D.J., Schenk, G.J., Rocca, M.A., Filippi, M., Geurts, J.J.G., Jonkman, L.E., 2019. Axonal degeneration as substrate of fractional anisotropy abnormalities in multiple sclerosis cortex. Brain 142, 1921–1937. https://doi.org/10.1093/brain/awz143
Rae-Grant, A., Day, G.S., Marrie, R.A., Rabinstein, A., Cree, B.A.C., Gronseth, G.S., Haboubi, M., Halper, J., Hosey, J.P., Jones, D.E., Lisak, R., Pelletier, D., Potrebic, S., Sitcov, C., Sommers, R., Stachowiak, J., Getchius, T.S.D., Merillat, S.A., Pringsheim, T., 2018. Practice guideline recommendations summary: Disease-modifying therapies for adults with multiple sclerosis. Neurology 90, 777–788. https://doi.org/10.1212/WNL.0000000000005347
Rahmanzadeh, R., Galbusera, R., Lu, P., Bahn, E., Weigel, M., Barakovic, M., Franz, J., Nguyen, T.D., Spincemaille, P., Schiavi, S., Daducci, A., la Rosa, F., Absinta, M., Sati, P., Bach Cuadra, M., Radue, E., Leppert, D., Kuhle, J., Kappos, L., Brück, W., Reich, D.S., Stadelmann, C., Wang, Y., Granziera, C., 2022. A New Advanced <scp>MRI</scp> Biomarker for Remyelinated Lesions in Multiple Sclerosis. Ann Neurol 92, 486–502. https://doi.org/10.1002/ana.26441
Rahmanzadeh, R., Lu, P.-J., Barakovic, M., Weigel, M., Maggi, P., Nguyen, T.D., Schiavi, S., Daducci, A., la Rosa, F., Schaedelin, S., Absinta, M., Reich, D.S., Sati, P., Wang, Y., Bach Cuadra, M., Radue, E.-W., Kuhle, J., Kappos, L., Granziera, C., 2021. Myelin and axon pathology in multiple sclerosis assessed by myelin water and multi-shell diffusion imaging. Brain 144, 1684–1696. https://doi.org/10.1093/brain/awab088

Reich, D.S., Lucchinetti, C.F., Calabresi, P.A., 2018. Multiple Sclerosis. New England Journal of Medicine 378, 169–180. https://doi.org/10.1056/NEJMra1401483

Rocca, M.A., Mondria, T., Valsasina, P., Sormani, M.P., Flach, Z.H., te Boekhorst, P.A., Comi, G., Hintzen, R.Q., Filippi, M., 2007. A Three-Year Study of Brain Atrophy after Autologous Hematopoietic Stem Cell Transplantation in Rapidly Evolving Secondary Progressive Multiple Sclerosis. American Journal of Neuroradiology 28, 1659–1661. https://doi.org/10.3174/ajnr.A0644

Roccatagliata, L., Rocca, M., Valsasina, P., Bonzano, L., Sormani, M., Saccardi, R., Mancardi, G., Filippi, M., 2007. The long-term effect of AHSCT on MRI measures of MS evolution: a five-year follow-up study. Multiple Sclerosis Journal 13, 1068–1070. https://doi.org/10.1177/1352458507076982

Rotstein, D.L., Healy, B.C., Malik, M.T., Chitnis, T., Weiner, H.L., 2015. Evaluation of No Evidence of Disease Activity in a 7-Year Longitudinal Multiple Sclerosis Cohort. JAMA Neurol 72, 152. https://doi.org/10.1001/jamaneurol.2014.3537

Rush, C.A., MacLean, H.J., Freedman, M.S., 2015. Aggressive multiple sclerosis: proposed definition and treatment algorithm. Nat Rev Neurol 11, 379–389. https://doi.org/10.1038/nrneurol.2015.85

Saccardi, R., Freedman, M., Sormani, M., Atkins, H., Farge, D., Griffith, L., Kraft, G., Mancardi, G., Nash, R., Pasquini, M., Martin, R., Muraro, P., 2012. A prospective, randomized, controlled trial of autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: a position paper. Multiple Sclerosis Journal 18, 825–834. https://doi.org/10.1177/1352458512438454

Samijn, J.P.A., 2006. Intense T cell depletion followed by autologous bone marrow transplantation for severe multiple sclerosis. J Neurol Neurosurg Psychiatry 77, 46–50. https://doi.org/10.1136/jnnp.2005.063883

Sharrack, B., Saccardi, R., Alexander, T., Badoglio, M., Burman, J., Farge, D., Greco, R., Jessop, H., Kazmi, M., Kirgizov, K., Labopin, M., Mancardi, G., Martin, R., Moore, J., Muraro, P.A., Rovira, M., Sormani, M.P., Snowden, J.A., 2020. Autologous haematopoietic stem cell transplantation and other cellular therapy in multiple sclerosis and immune-mediated neurological diseases: updated guidelines and recommendations from the EBMT Autoimmune Diseases Working Party (ADWP) and the Joint Accreditation Committee of EBMT and ISCT (JACIE). Bone Marrow Transplant 55, 283–306. https://doi.org/10.1038/s41409-019-0684-0

Shevchenko, J.L., Kuznetsov, A.N., Ionova, T.I., Melnichenko, V.Y., Fedorenko, D.A., Kurbatova, K.A., Gorodokin, G.I., Novik, A.A., 2015. Long-term outcomes of autologous hematopoietic stem cell transplantation with reduced-intensity conditioning in multiple sclerosis: physician's and patient's perspectives. Ann Hematol 94, 1149–1157. https://doi.org/10.1007/s00277-015-2337-8

Signoriello, E., Lus, G., Bonavita, S., Lanzillo, R., Saccà, F., Landi, D., Frau, J., Baroncini, D., Zaffaroni, M., Maniscalco, G.T., Curti, E., Sartori, A., Cepparulo, S., Marfia, G.A., Nicoletti, C.G., Carotenuto, A., Nociti, V., Caleri, F., Sormani, M.P., Signori, A., 2022. Switch from sequestering to anti-CD20 depleting treatment: disease activity outcomes during wash-out and in the first 6 months of ocrelizumab therapy. Multiple Sclerosis Journal 28, 93–101. https://doi.org/10.1177/13524585211005657

Snarski, E., Snowden, J.A., Oliveira, M.C., Simoes, B., Badoglio, M., Carlson, K., Burman, J., Moore, J., Rovira, M., Clark, R.E., Saiz, A., Hadj-Khelifa, S., Tan, J., Crescimanno, A., Musso, M., Martin, T., Farge, D., 2015. Onset and outcome of pregnancy after autologous haematopoietic SCT (AHSCT) for autoimmune diseases: A retrospective study of the EBMT autoimmune diseases

working party (ADWP). Bone Marrow Transplant 50, 216–220. https://doi.org/10.1038/bmt.2014.248

Sormani, M.P., Muraro, P.A., Saccardi, R., Mancardi, G., 2017a. NEDA status in highly active MS can be more easily obtained with autologous hematopoietic stem cell transplantation than other drugs. Multiple Sclerosis Journal 23, 201–204. https://doi.org/10.1177/1352458516645670

Sormani, M.P., Muraro, P.A., Schiavetti, I., Signori, A., Laroni, A., Saccardi, R., Mancardi, G.L., Capello, E., Uccelli, A., Bagigalupo, F., Repice, A.M., Portaccio, E., Barillaro, A., Russo, C., de Luca, G., Farina, D., Tartaro, A., Blanco, Y., Berenguer, J., Bresciani, P., Cuoghi, A., Zonari, P., 2017b. Autologous hematopoietic stem cell transplantation in multiple sclerosis: A meta-analysis. Neurology 88, 2115–2122. https://doi.org/10.1212/WNL.0000000000003987

Sucksdorff, M., Rissanen, E., Tuisku, J., Nuutinen, S., Paavilainen, T., Rokka, J., Rinne, J., Airas, L., 2017. Evaluation of the effect of fingolimod treatment on microglial activation using serial PET imaging in multiple sclerosis. Journal of Nuclear Medicine 58, 1646–1651. https://doi.org/10.2967/jnumed.116.183020

Sucksdorff, M., Tuisku, J., Matilainen, M., Vuorimaa, A., Smith, S., Keitilä, J., Rokka, J., Parkkola, R., Nylund, M., Rinne, J., Rissanen, E., Airas, L., 2019. Natalizumab treatment reduces microglial activation in the white matter of the MS brain. Neurol Neuroimmunol Neuroinflamm 6, 1–10. https://doi.org/10.1212/NXI.0000000000000574

Thebault, S., Lee, H., Bose, G., Tessier, D., Abdoli, M., Bowman, M., Berard, J., Walker, L., Rush, C.A., MacLean, H., Booth, R.A., Narayanan, S., Arnold, D.L., Tabard-Cossa, V., Atkins, H.L., Bar-Or, A., Freedman, M.S., 2020. Neurotoxicity after hematopoietic stem cell transplant in multiple sclerosis. Ann Clin Transl Neurol 7, 767–775. https://doi.org/10.1002/acn3.51045

Thebault, S., R. Tessier, D., Lee, H., Bowman, M., Bar-Or, A., Arnold, D.L., L. Atkins, H., Tabard-Cossa, V., Freedman, M.S., 2019. High serum neurofilament light chain normalizes after hematopoietic stem cell transplantation for MS. Neurology - Neuroimmunology Neuroinflammation 6, e598. https://doi.org/10.1212/NXI.0000000000000598

Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., Correale, J., Fazekas, F., Filippi, M., Freedman, M.S., Fujihara, K., Galetta, S.L., Hartung, H.P., Kappos, L., Lublin, F.D., Marrie, R.A., Miller, A.E., Miller, D.H., Montalban, X., Mowry, E.M., Sorensen, P.S., Tintoré, M., Traboulsee, A.L., Trojano, M., Uitdehaag, B.M.J., Vukusic, S., Waubant, E., Weinshenker, B.G., Reingold, S.C., Cohen, J.A., 2018a. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17, 162–173. https://doi.org/10.1016/S1474-4422(17)30470-2

Thompson, A.J., Baranzini, S.E., Geurts, J., Hemmer, B., Ciccarelli, O., 2018b. Multiple sclerosis. The Lancet 391, 1622–1636. https://doi.org/10.1016/S0140-6736(18)30481-1

Tintore, M., Arrambide, G., Otero-Romero, S., Carbonell-Mirabent, P., Río, J., Tur, C., Comabella, M., Nos, C., Arévalo, M.J., Anglada, E., Menendez, R., Midaglia, L., Galán, I., Vidal-Jordana, A., Castilló, J., Mulero, P., Zabalza, A., Rodríguez-Acevedo, B., Rodriguez, M., Espejo, C., Sequeira, J., Mitjana, R., de Barros, A., Pareto, D., Auger, C., Pérez-Hoyos, S., Sastre-Garriga, J., Rovira, A., Montalban, X., 2020. The long-term outcomes of CIS patients in the Barcelona inception cohort: Looking back to recognize aggressive MS. Multiple Sclerosis Journal 26, 1658–1669. https://doi.org/10.1177/1352458519877810

Tonietto, M., Poirion, E., Lazzarotto, A., Ricigliano, V., Papeix, C., Bottlaender, M., Bodini, B., Stankoff, B., 2022. Periventricular remyelination failure in multiple sclerosis: a substrate for neurodegeneration. Brain. https://doi.org/10.1093/brain/awac334

van Wijmeersch, B., Singer, B.A., Boster, A., Broadley, S., Fernández, Ó., Freedman, M.S., Izquierdo, G., Lycke, J., Pozzilli, C., Sharrack, B., Steingo, B., Wiendl, H., Wray, S., Ziemssen, T., Chung, L., Margolin, D.H., Thangavelu, K., Vermersch, P., 2020. Efficacy of alemtuzumab over

6 years in relapsing–remitting multiple sclerosis patients who relapsed between courses 1 and 2: Post hoc analysis of the CARE-MS studies. Multiple Sclerosis Journal 26, 1719–1728. https://doi.org/10.1177/1352458519881759

Vercellino, M., Marasciulo, S., Grifoni, S., Vallino-Costassa, E., Bosa, C., Pasanisi, M.B., Crociara, P., Casalone, C., Chiò, A., Giordana, M.T., Corona, C., Cavalla, P., 2022. Acute and chronic synaptic pathology in multiple sclerosis gray matter. Multiple Sclerosis Journal 28, 369–382. https://doi.org/10.1177/13524585211022174

Willison, A.G., Ruck, T., Lenz, G., Hartung, H.P., Meuth, S.G., 2022. The current standing of autologous haematopoietic stem cell transplantation for the treatment of multiple sclerosis. J Neurol 269, 3937–3958. https://doi.org/10.1007/s00415-022-11063-5

Wundes, A., Bowen, J.D., Kraft, G.H., Maravilla, K.R., McLaughlin, B., von Geldern, G., Georges, G., Nash, R.A., Lu, J.-Q., 2017. Brain pathology of a patient 7 years after autologous hematopoietic stem cell transplantation for multiple sclerosis. J Neurol Sci 373, 339–341. https://doi.org/10.1016/j.jns.2017.01.016

Zecca, C., Bovis, F., Novi, G., Capobianco, M., Lanzillo, R., Frau, J., Repice, A.M., Hakiki, B., Realmuto, S., Bonavita, S., Curti, E., Brambilla, L., Mataluni, G., Cavalla, P., di Sapio, A., Signoriello, E., Barone, S., Maniscalco, G.T., Maietta, I., Maraffi, I., Boffa, G., Malucchi, S., Nozzolillo, A., Coghe, G., Mechi, C., Salemi, G., Gallo, A., Sacco, R., Cellerino, M., Malentacchi, M., de Angelis, M., Lorefice, L., Magnani, E., Prestipino, E., Sperli, F., Brescia Morra, V., Fenu, G., Barilaro, A., Abbadessa, G., Signori, A., Granella, F., Amato, M.P., Uccelli, A., Gobbi, C., Sormani, M.P., 2019. Treatment of multiple sclerosis with rituximab: A multicentric Italian-Swiss experience. Mult Scler 1352458519872889. https://doi.org/10.1177/1352458519872889

Zhukovsky, C., Sandgren, S., Silfverberg, T., Einarsdottir, S., Tolf, A., Landtblom, A.-M., Novakova, L., Axelsson, M., Malmestrom, C., Cherif, H., Carlson, K., Lycke, J., Burman, J., 2021. Autologous haematopoietic stem cell transplantation compared with alemtuzumab for relapsing–remitting multiple sclerosis: an observational study. J Neurol Neurosurg Psychiatry 92, 189–194. https://doi.org/10.1136/jnnp-2020-323992

Zinger, N., Ponath, G., Sweeney, E., Nguyen, T.D., Lo, C.H., Diaz, I., Dimov, A., Teng, L., Zexter, L., Comunale, J., Wang, Y., Pitt, D., Gauthier, S.A., 2022. Dimethyl Fumarate Reduces Inflammation in Chronic Active Multiple Sclerosis Lesions. Neurology - Neuroimmunology Neuroinflammation 9, e1138. https://doi.org/10.1212/NXI.0000000000001138

Zivadinov, R., Ramasamy, D.P., Vaneckova, M., Gandhi, S., Chandra, A., Hagemeier, J., Bergsland, N., Polak, P., Benedict, R.H.B., Hojnacki, D., Weinstock-Guttman, B., 2017. Leptomeningeal contrast enhancement is associated with progression of cortical atrophy in MS: A retrospective, pilot, observational longitudinal study. Multiple Sclerosis 23, 1336–1345. https://doi.org/10.1177/1352458516678083