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Ocrelizumab in MS patients with persistence of disease activity after alemtuzumab: A multi-center Italian study

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Abstract

Background: The reason why some multiple sclerosis (MS) patients show disease activity after alemtuzumab (ALM) is still unclear, but ocrelizumab (OCR) could represent an interesting sequential therapeutic approach.

Objectives: To investigate safety and efficacy of OCR in MS patients with disease activity after two ALM courses.

Methods: Observational retrospective multi-centers Italian cohort study.

Results: Seventy-two subjects were included. Mean follow-up (FU) was 2.4 (±1) years. Forty-five patients (62.5%) experienced at least one adverse event (AE), with infections accounting for 96.7% of cases. A reduction in total lymphocytes was observed between OCR start and 6 months FU, driven by BCD19+ lymphocytes depletion ($p < 0.001$). Immunoglobulin M (IgM) levels decreased between OCR start and 6 months FU ($p < 0.001$). At 2-year FU, relapse, magnetic resonance imaging (MRI) activity and disability worsening-free survival were 92.1%, 90.8%, and 89.2%. The evidence of inflammatory activity between the two ALM courses was associated with higher risk of relapse, MRI activity, and NEDA-3 status loss in relapsing-remitting multiple sclerosis (RRMS; $p = 0.02$, $p = 0.05$, $p = 0.01$, respectively).

Conclusions: OCR after two ALM courses seemed to be safe and effective. Early IgM hypogammaglobulinemia occurred in a high proportion of patients. The evidence of inflammatory activity between ALM courses seemed to increase the risk of MS re-activation on OCR treatment.

Keywords: Multiple sclerosis, alemtuzumab, ocrelizumab, induction, therapy, safety, efficacy

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Introduction

In recent times, there has been a significant shift toward the early employment of high-efficacy therapies in multiple sclerosis (MS) treatment,[1] diverging from the escalating strategy recommended over the last two decades.[2] Early adoption of induction together with high-efficacy disease-modifying therapies may offer the most favorable risk–benefit profile, particularly for young patients with highly active relapsing-remitting (RR) MS.[1] Among approved drugs, alemtuzumab (ALM) is one of the most effective induction therapies against MS.[3,4] Data from the extension phases of ALM clinical trials showed excellent long-term efficacy even if 68% and 60% of patients enrolled in CARE-MS I and CARE-MS II needed a re-treatment with ALM during the 3 years following the last ALM cycle.[5,6] The reason why some MS patients show persistence of inflammatory disease activity after ALM treatment is still matter of debate. The development of neutralizing antidrug antibodies[7] does not seem to be sufficient to explain disease persistence after the first two ALM courses. Alternatively, a B-cell driven mechanism has been suggested, particularly in paradoxical disease

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re-activation, where single case reports described the good outcome achieved after the use of anti-CD20 therapies.[8] Therefore, very little is known about the immunological mechanisms underlying the persistence of disease activity after ALM, as well as about the development of secondary autoimmune complications,[9] for which a targeted B-cell depletion strategy has been recently suggested.[10] Furthermore, although a role of ALM in reducing risk of secondary progression has been hypothesized,[11] this might be limited to patients showing residual inflammatory activity. In such scenario, treatment with ocrelizumab (OCR) could represent an interesting therapeutic approach.

The aims of this study were to investigate (1) safety and (2) efficacy of treatment with OCR in MS patients who experienced persistence of inflammatory activity and/or disability progression after the first two ALM courses.

Materials and methods

This is an observational retrospective multi-centers cohort study conducted at different MS Centers in Italy. MS patients who started OCR after the first two ALM courses from June 2019 to July 2023 and had at least 6 months follow-up (FU) after OCR initiation was consecutively included. RR and progressive (relapsing and no relapsing, from now on "RP" and "PnoR," respectively) courses were defined according to Lublin et al.[12] From March to June 2020, due to coronavirus disease 2019 (COVID-19) pandemic, OCR was administered following a tailored approach evaluating the profile risk of each patient, according to international indications.[13,14] Given the real-life and multi-center nature of the study, in order to minimize the heterogeneity of data, we used the reference values derived from the American Board of Internal Medicine (ABIM)[15] to define when a laboratory variable fell into a pathological range (for TCD4+ normal range: 530–1570 cell/mm^3, for TCD8+ normal range: 430–1060 cell/mm^3, for BCD19+ normal range: 208–590 cell/mm^3, respectively; IgG normal range: 800–1500 g/L, IgA normal range: 90–325 g/L, IgM normal range: 45–150 g/L, respectively).

Study endpoints

The primary objective of our study was to assess safety of OCR treatment after two ALM courses in terms of (1) IARs, (2) infections and cancer, (3) secondary autoimmune events, (4) laboratory changes in lymphocyte profiling, immunoglobulin concentration, thyroid function, and autoimmunity. As IARs are concerned, all OCR patients performed pre-medication according to the Italian SPC:[16] 100 mg of methylprednisolone (or equivalent) intravenously approximately 30 minutes before each infusion and antihistamine about 30–60 minutes before each infusion. The secondary aims were to determine efficacy of OCR therapy in terms of (1) time-to-first relapse (defined as "new or worsening symptoms attributable to MS, preceded by 30 days of stability, lasting for more than 24 hours, not associated with fever, and leading to $\geqslant 0.5$ EDSS points increase compared to a prior visit or $\geqslant 2$ points increase in one EDSS functional system, or $\geqslant 1$ point increase in two EDSS functional systems (excepting bladder and cognitive changes")),[17] time-to-first evidence of magnetic resonance imaging (MRI) activity (defined as "evidence of new T2/FLAIR lesion/s and/or gadolinium enhancing lesions and/or enhancement of pre-existing T2/FLAIR lesion/s"), time-to-confirmed disability worsening (defined as "an increase of at least 1.5 points for baseline EDSS of 0, an increase of at least 1.0 point if baseline EDSS was 1–5.5 points or an increase of at least 0.5 points if baseline EDSS was >5.5 points")[18] and time-to-first evidence of disease activity (according to the NEDA-3 definition); (2) clinical predictors of inflammatory activity (relapse and/or MRI activity) and disability progression during OCR treatment. Routinary clinical evaluations were performed every 3–6 months during OCR FU. Baseline brain MRI (acquired within 3 months before OCR start) was the pre-treatment reference scan. Where available, also data from spinal cord MRIs were used. For the inclusion in this study, at least one brain MRI per year after OCR start was required. Out of the total cohort, 56 (77.8%) patients performed brain MRI every 6 months, 16 (22.2%) performed MRI once a year. Patients who clinically relapsed[17] performed additional brain or spinal cord MRI based on their symptoms. The application of the definition of "relapse," associated with the careful exclusion of concomitant potentially confounding conditions (fever, metabolic abnormalities . . .),[17] associated with the radiological demonstration of new inflammatory activity at the time of the clinical relapse, minimized the risk of including "pseudorelapses" in this study. All the analyses concerning efficacy aims were performed (1) on the global cohort, and, as sensitivity analysis, (2) on RRMS and (3) on progressive MS (PMS), with the latest one originated from the inclusion of PnoR and RP patients together, thus considering as a whole all patients who showed disability progression.

Statistical analysis

Analysis of covariance (ANCOVA) for repeated measures corrected for gender, age, wash-out between

the second ALM course and OCR start was used to evaluate changes in total lymphocytes, lymphocytes subsets, and immunoglobulin levels between OCR start and 6-, 12-, and 24-month FU (Bonferroni corrected). The probability of relapse-free survival, MRI activity-free survival, disability worsening-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. A two-sided $p < 0.05$ was used for statistical significance (SPSS 23, IBM; version 23.0).

The Ethics Committee of IRCCS Ospedale Policlinico San Martino (Genoa) approved the study (CE 2460PRNO240220 "Alem-Stop" 73/2020 10344), conducted in accordance with the ethical principles of the Declaration of Helsinki. All participants provided consent to use their medical history for publication.

Data are available upon reasonable request.

Results

Data about 72 consecutive MS patients (57 RR, 10 RP, 5 PnoR who started OCR after the first two ALM courses were collected. Mean FU from OCR start was 2.4 (±1) years. Demographic- and disease-related characteristics of patients are reported in Table 1.

Table 1. Demographic- and disease-related features of study population.

Patients, *n*	72
Age at OCR start, mean (SD), years	39.1 (9.2)
Gender, female (%)	63.9
MS phenotype at OCR start, %	
RR	79.2
RP	13.9
PnoR	6.9
DMTs pre-ALM	
Naïve, %	12.5
I-line therapy, %	27.8
Fingolimod, %	40.3
Natalizumab, %	23.6
Cumulative no of relapses after ALM, mean	69
No. of new T2 lesions at MS reactivation after ALM, mean (SD)	3 (3.2)
No. of Gd+ lesions at MS reactivation after ALM, mean (SD)	1 (2)
Time between last ALM and OCR start, mean (SD), months	29 (11.6)
Disease duration at OCR start, mean (SD), years	12.4 (6.7)
FU duration from OCR start, mean (SD), years	2.4 (1)
EDSS at ALM start, median (IQR)	4 (1–8)
EDSS at OCR start, median (IQR)	3.5 (0–8)
Reason of switch to OCR	
Inflammation (relapse and/or MRI activity), *n* (%)	62 (86.1)
Disability progression, *n* (%)	10 (13.9%)

OCR: ocrelizumab; MS: multiple sclerosis; RR: relapsing remitting; RP: relapsing progressing; PnoR: progressive no-relapsing; DMT: disease modifying therapy; ALM: alemtuzumab; I line: first line; FU: follow-up; EDSS: Expanded Disability Status Scale; IQR: interquartile range; MRI: magnetic resonance imaging.

Primary aims

Infusion-associated reactions. Ten (13.9%) and 1 (1.4%) out of 72 MS patients developed infusion-associated reactions (IARs) at first and/or second inductive OCR infusion, respectively. None of them was "severe," 10 were "moderate" ($n = 7$ skin rash, $n = 3$ headache), and 1 was "mild" (headache) in severity.[19] An antihistamine drug and acetaminophen were administered with rapid and complete recovery. No IARs were notified at following OCR infusions.

Infections. Forty-five patients (62.5%) experienced at least one adverse event (AE) while on OCR therapy. Mean (SD) number of AEs per patient was 1 (1–2). Infections accounted for the vast majority (96.7%) of total AEs. Patients with progressive MS (PnoR + RP) had a slightly increased, although not significant, incidence of AEs (62.8% vs 58.4%). Most patients (84.3%) experienced the first AE within the first year of treatment. In two patients (2.8%), a diagnosis of neoplasm was reported: colic cancer ($n = 1$, at +370 days from first OCR course, with no lymph nodal or metastatic involvement) and cervical cancer (CIN2 $n = 1$, +457 days). Both patients achieved complete remission of their neoplastic comorbidity at their maximum FU (3.2 and 2.8 years, respectively).

No infectious events required hospitalization nor fulfilled other criteria for serious AEs.[20]

Two patients required hospitalization due to safety issues ($n = 1$ for colic cancer, $n = 1$ for appendicectomy).

AEs and serious AEs are detailed in Table 2.

Secondary autoimmunity. One patient (1.4%) received diagnosis of thyroiditis a few days after the

Table 2. Primary aims: safety.

	Total cohort	RR	PnoR + RP
Any adverse events, *n* (%)	61 (62.5)	35 (58.4)	26 (62.8)
Serious infusion associated reactions, *n* (%)	0	0	0
Adverse events leading to OCR discontinuation, *n* (%)	1(1.4)[a]	1 (1.7)	0
Adverse events leading to hospitalization, *n* (%)	2[b] (2.8)	0	2 (13.3)
Number of adverse events per subject, median (IQR)	1 (1–2)	1 (1–2)	1 (1–2)
Pregnancy leading to OCR discontinuation, *n* (%)	1 (1.4)	1 (1.7)	0
Time from first OCR infusion, days, mean (range)	194 (67–416)	145 (67–404)	221 (68–487)
Infectious adverse events, *n* (%)	59 (59.7)	40 (56.2)	19 (60.7)
Pneumonia	1	1	0
Upper respiratory tract infection	35	25	10
Lower urinary tract infection	12	8	4
HSV1 reactivation	7	4	3
VZV reactivation	3	2	1
Appendicectomy	1	0	1
Neoplasm, *n* (%)	2[c] (2.8)	1 (1.7)	1 (6.7)
Death, *n* (%)	0	0	0

RR: relapsing remitting; PnoR: PnoR: progressive no-relapsing; RP: relapsing progressing; OCR: ocrelizumab; IQR: interquartile range; HSV1: herpes simplex virus type 1; VZV: varicella-zoster virus.
[a]Colic cancer.
[b]Colic cancer, appendicectomy.
[c]Colic cancer, cervical intraepithelial neoplasia grade 2 (CIN2).

first inductive OCR infusion. None of the patients developed other autoimmune complications during OCR treatment.

At 6- and 12-month FU, a decrease in the percentage of MS patients who showed thyroid stimulating hormone (TSH) abnormalities (hypo/hyper), anti-TSH antibodies positivity, and anti-TPO antibodies positivity was observed (data available for $n = 40$ patients). Figure 1 shows the percentage of patients with TSH abnormalities, anti-TSH and anti-TPO antibodies positivity at OCR start, 6- and 12-month FU.

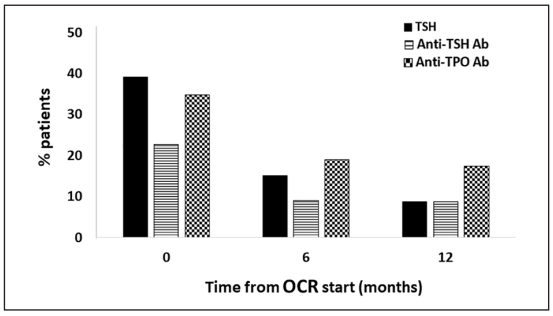

Figure 1. Proportion of patients with TSH abnormalities, anti-TSH and anti-TPO antibodies positivity at OCR start and during the follow-up.
For TSH (hypo/hyper): 39.1%, 15.1%, 8,7% at OCR start, 6- and 12-months FU, respectively; for anti-TSH antibodies positivity: 22.7%, 9.1%, 8,7% at OCR start, 6- and 12-month FU, respectively; for anti-TPO antibodies positivity: 34.8%, 19%, 17.4% at OCR start, 6- and 12-month FU, respectively. One patient candidate for thyroidectomy after II ALM course who started OCR in the meanwhile due to a severe relapse presented a rapid recovery of thyroid function, and at 6 months FU, also stopped any thyroid-related treatment.

Laboratory abnormalities

Lymphocyte profiling. Considering baseline, 6- and 12-month FU after OCR start, data about lymphocyte count were available for 33 (45.8%) MS patients. None of them showed lymphopenia at OCR start. A significant decrease in total lymphocytes count was observed between OCR start and 6 months FU (1648.5 ± 841.2 cell/mm^3 vs 1178 ± 392.9 cell/mm^3, mean difference = 470.5, 95% CI (97.6, 843.6), $p = 0.010$) and between OCR start and 12 months FU (1648.5 ± 841.2 cell/mm^3 vs 1285.6 ± 517.4 cell/mm^3, mean difference = 362.9, 95% confidence interval (CI) = [11.3, 714.5], $p = 0.041$).

Twenty-four (72.7%) MS patients showed TCD4+ lymphopenia at OCR start (363.2 ± 15.4 cell/mm^3). No significant differences in TCD4+ lymphocytes were observed, although a trend toward TCD4+ cells repopulation was evident (OCR start:

448.7 ± 37.5 cell/mm^3; 6 months FU: 486.5 ± 33.8 cell/mm^3; 12 months FU: 519.8 ± 35.4 cell/mm^3).

Eleven (33.3%) MS patients showed TCD8+ lymphopenia at OCR start (165.2 ± 15.4 cell/mm^3). No significant differences in TCD8+ lymphocytes were observed (OCR start: 302.1 ± 28.1 cell/mm^3; 6 months FU: 324.9 ± 29.9 cell/mm^3; 12 months FU: 318.7 ± 28.7 cell/mm^3).

None of MS patients showed BCD19+ lymphopenia at OCR start, while 8 (24.2%) showed BCD19+ lymphocytosis. A significant decrease in BCD19+ lymphocytes count was observed between OCR start and 6 months FU (415.9 ± 46.1 cell/mm^3 vs 36.8 ± 10.5 cell/mm^3, mean difference = 379.1, 95% CI = [264.3, 493.9], $p < 0.001$) and between OCR and 12 months FU (36.8 ± 10.5 cell/mm^3 vs 28.3 ± 8.5 cell/mm^3, mean difference = 37.6, 95% CI = [269.3, 505.9], $p < 001$). This finding was confirmed also MS patients who had BCD19+ lymphocytosis at OCR start ($p < 0.05$).

A subgroup analysis was performed on MS patients in which immunophenotype was available and reached 2-year FU ($n = 16$). A significant increase in TCD4+ and TCD8+ lymphocytes was observed between 12- and 24-month FU (532.5 ± 47.7 cell/mm^3 vs 634.1 ± 49.8 cell/mm^3, mean difference = –101.6, 95% CI = [–263.1, 59.9], $p < 0.05$ for TCD4+;: 328.7 ± 31.7 cell/mm^3 vs 429.5 ± 11.7 cell/mm^3, mean difference = –100.8, 95% CI = [–259.6, 57.4], $p < 0.05$ for TCD8+). BCD19+ cells depletion was maintained at 24 months FU (no significant differences between 6–12 and 24 months FU; between OCR start and 24 months FU $p < 0.001$).

Immunoglobulin levels. None of the patients showed hypo-IgG, hypo-IgA, nor hypo-IgM at OCR start. No significant differences in IgG and IgA levels were observed at 6- and 12-month FU. A significant decrease in IgM levels was observed between OCR start and 6 months FU (0.9 ± 0.1 g/L vs 0.6 ± 0.1 g/L, mean difference = 0.345, 95% CI = [0.2, 0.5], $p < 0.001$), stable at 12 months FU (0.6 ± 0.1 g/L). At OCR start, 15% patients had hypo-IgM (0.75 ± 0.1 g/L) and 48.1% at 6- and 12-month FU.

Lymphocyte profiling and Immunoglobulins levels are detailed in Table 3 and Figure 2.

No significant correlations between TCD4+, TCD8+ lymphopenia, and Ig (IgG, IgA, and IgM) levels at OCR follow-ups and infection rates were detected. Specifically, 13/58 (22.4%) patients who developed infectious complications of any type and grade showed TCD4+ or TCD8+ lymphopenia before or at the moment of infection development. As expected, all of them were BCD19+ cells depleted. As Ig levels are concerned, 27/58 (46.6%), 2/58 (3.4%), and 0 (0%) patients who developed infectious complications of any type and grade showed evidence of IgM, IgG, and IgA hypogammaglobulinemia at 6 and 12 months FU after OCR start, respectively.

Secondary aims

Figure 3 (a) to (h) reports the results of the efficacy aims for the whole cohort, RRMS, and PMS (PnoR + RP) separately.

Whole cohort. At 2-year FU, 92.1% of patients were free of relapses (see Figure 3 (a)). At 2-year FU, 90.8% of patients were free of MRI activity (see Figure 3 (b)). At 2-year FU, 89.2% patients were free of disability worsening (see Figure 3 (c)). At 2-year FU, 79.9% of patients achieved NEDA-3 status (see Figure 3 (d)).

Subgroup analysis: RRMS and PMS. At 2-year FU, 92.1% of patients with RRMS were free of relapses. No PMS patients showed relapses during OCR treatment (see Figure 3 (e)). Five RRMS patients had a single relapse at +14, +12, +16, +18, and +10 months from OCR start. Out of them, one patient (EDSS increase +2.5) did not fully recover (6 months confirmed disability increase of +1 EDSS point).

At 2-year FU, 90.8% of patients with RRMS were free of MRI activity. No PMS patients showed evidence of MRI activity during OCR (see Figure 3 (f)). Two RRMS patients (3.5%) showed isolated MRI activity in absence of clinical correlates at +24 and +6 months from OCR start, respectively.

At 2-year FU, 94% of patients with RRMS and 72.7% patients with PMS were free of disability worsening, respectively (see Figure 3 (g)). At 2-year FU, NEDA-3 percentages were 82.1% and 72.7% for patients with RRMS and PMS, respectively (see Figure 3 (h)).

Table 4 reports the results of the univariate and multivariate analyses of clinical factors influencing efficacy for the whole cohort and RRMS subgroup.

In the whole cohort, higher EDSS at OCR initiation and disability worsening after ALM were associated with increased risk of progression during OCR ($p = 0.02$ and $p = 0.005$, respectively). At multivariate

Table 3. Lymphocyte counts and immunoglobulin levels during OCR therapy.

Total cohort	
Baseline	
Available, *n*	33
Total lymphocytes (cell/mm^3), mean (SD)	1648.52 (841.2)
BCD19+ lymphocytes (cell/mm^3), mean (SD)[a]	415.9 (46.2)
TCD4+ lymphocytes (cell/mm^3), mean (SD)[b]	448.7 (37.5)
TCD8+ lymphocytes (cell/mm^3), mean (SD)[c]	302.1 (28.1)
Available, *n*	27
IgG (g/L), mean (SD)[d]	8.9 (0.3)
IgA (g/L), mean (SD)[e]	1.9 (0.5)
IgM (g/L), mean (SD)[f]	0.9 (0.1)
6 months	
Available, *n*	33
Total lymphocytes (cell/mm^3), mean (SD)	1178 (392.9)
BCD19+ lymphocytes (cell/mm^3), mean (SD)[a]	36.8 (10.5)
TCD4+ lymphocytes (cell/mm^3), mean (SD)[b]	486.5 (33.8)
TCD8+ lymphocytes (cell/mm^3), mean (SD)[c]	324.9 (29.9)
Available, *n*	27
IgG (g/L), mean (SD)[d]	9.3 (0.4)
IgA (g/L), mean (SD)[e]	1.6 (0.1)
IgM (g/L), mean (SD)[f]	0.6 (0.1)
12 months	
Available, *n*	33
Total lymphocytes (cell/mm^3), mean (SD)	1285.7 (517.4)
BCD19+ lymphocytes (cell/mm^3), mean (SD)[a]	28.3 (8.5)
TCD4+ lymphocytes (cell/mm^3), mean (SD)[b]	519.8 (35.4)
TCD8+ lymphocytes (cell/mm^3), mean (SD)[c]	318.7 (28.6)
Available, *n*	27
IgG (g/L), mean (SD)[d]	9.2 (0.4)
IgA (g/L), mean (SD)[e]	1.7 (0.1)
IgM (g/L), mean (SD)[f]	(0.1)

OCR: ocrelizumab.
From American Board of Internal Medicine (ABIM)[15]:
[a]BCD19+ normal range: 208–590 cell/mm^3.
[b]TCD4+ normal range: 530–1570 cell/mm^3.
[c]TCD8+ normal range: 430–1060 cell/mm3.
[d]IgG normal range: 800–1500 g/L.
[e]IgA normal range: 90–325 g/L.
[f]IgM normal range: 45–150 g/L.

analyses, none of the previous variables were identified as independent predictors. In the whole cohort, the evidence of inflammatory activity (clinical relapse and/or MRI activity) between the two ALM courses was associated with higher risk of MRI activity during OCR ($p=0.03$), while a trend was noted for risk of clinical relapse ($p=0.08$). The same variable was associated with higher risk of relapse, MRI activity, and NEDA-3 status loss in the RRMS subgroup ($p=0.02$, $p=0.05$, $p=0.01$, respectively).

Discussion

We herein provide safety and effectiveness data about OCR treatment in MS patients who showed persistence of disease activity, both in terms of inflammation and disability progression after the first two ALM courses, retrospectively followed in a real-world multi-center Italian setting for a mean FU of almost 2.5 years.

The treatment strategy after ALM is still a matter of debate, with extension studies of pivotal trials that reported efficacy and safety of additional ALM courses[5,6,21] and only case reports that described outcomes of switching to other disease modifying therapies (DMTs), mainly anti-CD20 depleting agents.[8,22–25] In our study, MS severity after ALM in some patients, the radiological and immunological characteristics at the re-activation in others (multiple ring-enhancing lesions, BCD19+ cells lymphocytosis), the rapid disability worsening, and the concomitance of likely B-cell-mediated secondary autoimmunity after ALM[26] have been the main reasons behind the switch to OCR.

Regarding IARs, we did not observe any critical issues. In most cases (10.9%), OCR-related IARS were limited to inductive infusions, moderate in severity and completely resolved with common symptomatic therapies. In line with phase 2 and 3 clinical trials[27] and the few real-world studies available,[28,29] most infectious events occurred within the first year of treatment[28] and a slightly increased risk of AEs among real-life PMS patients was noted in our study.[28,30] Upper respiratory tract infections represented the most frequently observed AEs. None of the patients developed severe forms of COVID-19 disease. These reassuring findings seem to find a possible explanation in the dynamics of lymphocyte subpopulations during OCR. Indeed, the significant reduction in total lymphocyte count observed at 6- and 12-month FU was clearly driven, as expected, by B-cell depletion. Although CD20+ T cells have been

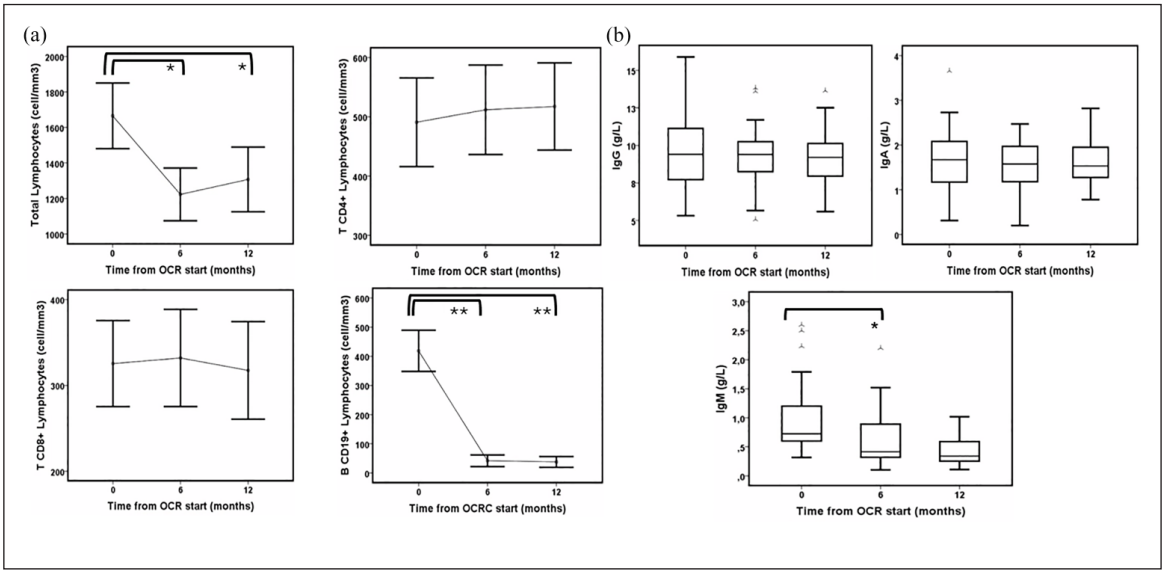

Figure 2. (a) Total lymphocytes, lymphocytes subpopulations and (b) immunoglobulins at OCR start, 6 and 12 months FU.

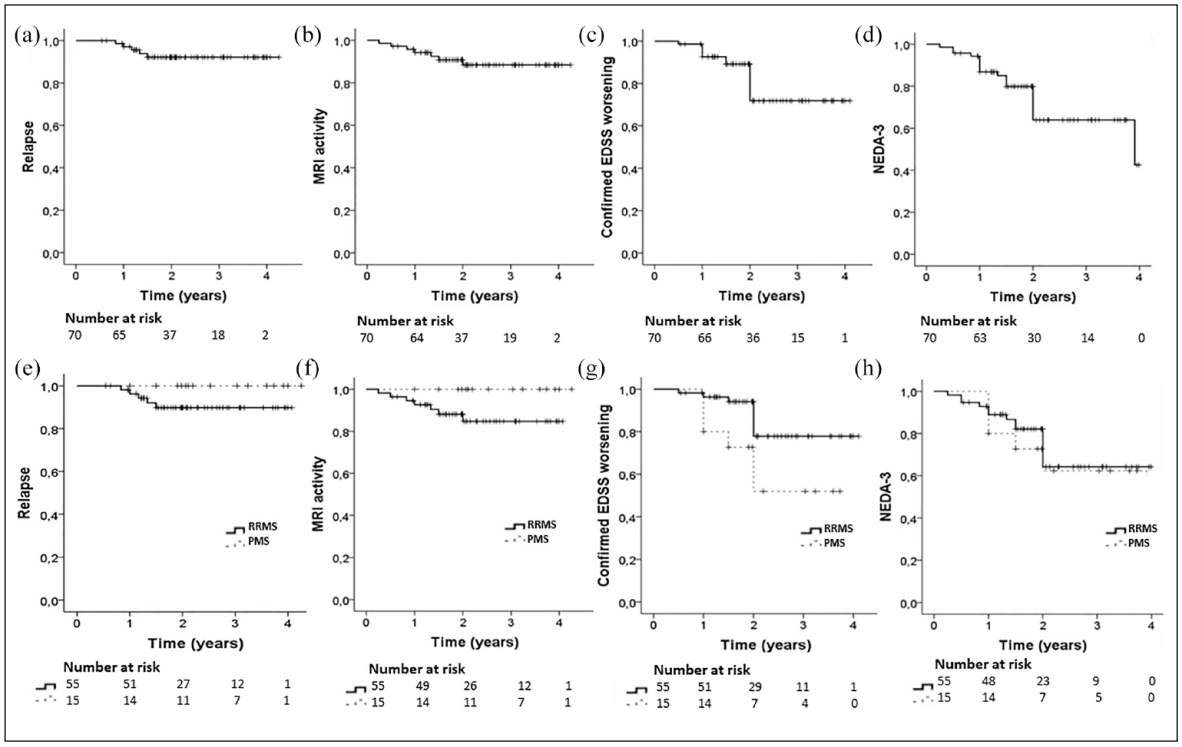

Figure 3. (a–h) Results of the efficacy aims for the whole cohort, RRMS, and PMS (PnoR + RP) are reported separately.

recently described as targets of OCR treatment,[31,32] we did not observe a significant decrease in T cells counterparts during OCR therapy. Conversely, although these data were available only for patients' subgroups, we observed a trend toward an increase in TCD4+ and TCD8+ lymphocytes during the first year FU, which became significant between 1- and 2-year FU.

Conversely, we observed a significant decrease in IgM levels between OCR start and 6 months FU. Our data exceed the percentage of IgM

Table 4. Univariate and multivariate analyses of factors associated with efficacy (whole cohort and RRMS subgroup).

	Disability worsening		MRI-inflammatory activity		Relapse		NEDA-3 status	
Total cohort ($n=72$)								
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (at OCR start)	1.01 (0.96–1.07)	0.54	0.96 (0.88–1.05)	0.43	0.01 (0.92–0.11)	0.87	1.01 (0.96–1.07)	0.56
Gender, female/male	0.35 (0.11–1.05)	0.06	0.87 (0.19–3.91)	0.86	0.93 (0.16–5.59)	0.94	0.53 (0.22–1.28)	0.16
Disease duration	1.06 (0.99–1.14)	0.09	0.93 (0.81–1.07)	0.38	0.94 (0.79–1.11)	0.46	1.03 (0.96–1.09)	0.39
EDSS (at OCR start)	1.42 (1.06–1.92)	**0.02**	0.82 (0.55–1.21)	0.31	0.87 (0.56–1.36)	0.55	1.16 (0.93–1.45)	0.18
Switch to OCR (inflammatory activity/progression)	0.17 (0.05–0.59)	**0.005**	—[a]	—[a]	—[a]	—[a]	0.45 (0.14–1.41)	0.17
Wash-out II ALM course-OCR start	0.98 (0.93–1.04)	0.58	1.01 (0.94–1.08)	0.82	0.99 (0.91–1.08)	0.91	0.99 (0.95–1.04)	0.81
Time interval MS re-activation after II ALM-OCR start	0.99 (0.88–1.11)	0.86	0.87 (0.67–1.12)	0.29	0.91 (0.71–1.17)	0.48	0.96 (0.86–1.07)	0.47
Inflammatory activity between I and II ALM courses (yes/no)	1.34 (0.45–4.01)	0.60	0.09 (0.01–0.79)	**0.03**	1.15 (0.02–1.31)	0.08	0.53 (0.21–1.31)	0.17
RRMS ($n=57$)								
Age (at OCR start)	1 (0.93–1.08)	0.96	0.98 (0.89–1.08)	0.73	1.03 (0.93–1.14)	0.52	1.01 (0.94–1.07)	0.84
Gender, female/male	0.31 (0.07–1.29)	0.11	0.75 (0.17–3.37)	0.75	0.81 (0.14–4.88)	0.82	0.54 (0.18–1.53)	0.24
Disease duration	1.08 (0.98–1.19)	0.10	0.96 (0.84–1.11)	0.61	0.97 (0.82–1.14)	0.72	1.03 (0.96–1.12)	0.39
EDSS (at OCR start)	1.27 (0.84–1.89)	0.25	0.98 (0.63–1.52)	0.92	1.07 (0.64–1.79)	0.79	1.12 (0.83–1.53)	0.46
Switch to OCR (inflammatory activity/progression)	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Wash-out II ALM course-OCR start	0.95 (0.88–1.02)	0.18	1.01 (0.94–1.07)	0.87	0.99 (0.91–1.08)	0.87	0.98 (0.94–1.03)	0.53
Time interval MS re-activation after II ALM-OCR start	0.95 (0.78–1.15)	0.58	0.90 (0.71–1.14)	0.38	0.94 (0.75–1.17)	0.56	0.92 (0.79–1.07)	0.29
Inflammatory activity between I and II ALM courses (yes/no)	0.68 (1.17–2.74)	0.58	0.08 (0.01–0.64)	**0.02**	0.12 (0.01–1.07)	**0.05**	0.26 (0.08–0.78)	**0.01**

RRMS: relapsing-remitting multiple sclerosis; MRI: magnetic resonance imaging; NEDA-3: no evidence of disease activity; *n*: number; HR: hazard ratio; CI: confidence interval; OCR: ocrelizumab; EDSS: Expanded Disability Status Scale; ALM: alemtuzumab; MS: multiple sclerosis.
[a]No MS patients who switched for disease progression exhibited MRI inflammatory activity and/or relapses.
[b]No RRMS patients switched for disability progression.

hypogammaglobulinemia previously reported.[28] Nevertheless, the fact that ALM may change immunoglobulin levels (IgM more than IgG) in peripheral blood of MS patients, has been previously described.[33] The ALM-induced anti-inflammatory environment[33] and the impact of ALM on CD25+ long-lived plasma cells[34] have been suggested as possible explanations for delayed hypogammaglobulinemia after ALM. Speculatively, we might hypothesize a combined effect of ALM and OCR in the development of IgM hypogammaglobulinemia in our cohort. Notably, no significant correlations between TCD4+, TCD8+ lymphopenia and Ig (IgG, IgA, and IgM) levels at OCR follow-ups and infection rates were detected in our study. However, the relatively short FU may not have allowed to observe the impact of IgM hypogammaglobulinemia on IgG levels and the correspondent, although debated,[30] infectious risk throughout OCR treatment.

It is known that ALM causes rapid B-cell depletion with recovery to normal values after 6 months followed by a further rise of mainly naive B cells associated with a more pronounced T-reg phenotype.[9,35–37] B-cell hyper population, when a complete and appropriate T-cell regulation is still lacking, might predispose ALM-treated patients to secondary autoimmunities.[35,37] In such a scenario, a targeted B-cell depletion strategy has been recently attempted in a very small cohort of patients.[10] Our study seemed to confirm these preliminary findings. Indeed, no patients developed secondary autoimmune complications during established OCR treatment. Furthermore, a beneficial effect of OCR was evident in patients with pre-existing ALM-related autoimmune thyroid disease. Unfortunately, the lack of a control group of patients re-treated with ALM represents a limitation of this study. Nevertheless, an indirect comparison may be made by using data from the extension phases of ALM pivotal trials[38] where an undoubtedly higher incidence of thyroid autoimmunity (the most common after ALM) at years 3–6 has been reported (in particular, an exposure-adjusted incidence rate per 100 patient-years of 10%).

As far as efficacy is concerned, in the RRMS population we observed that, at 2-year FU, 92.1%, 90.8%, and 94% patients were free of relapses, MRI activity, and disability worsening, respectively, with an overall percentage of patients achieving NEDA-3 of 82.1%. As per the progressive cohort (including both PnoR and RP MS patients), we observed a sustained beneficial effect of OCR treatment in terms of disability worsening and NEDA-3 status at 2-year FU (72.7% for both). Our findings are in line with real-world evidence on OCR efficacy in RRMS,[28,29] while we found a better performance of OCR in PMS patients than that described in a recent paper by Lorefice et al.[29] The different composition of study population, fully characterized by primary progressive (PP) MS patients, may explain the discrepancy with respect to our study, in which 10/15 (66.7%) PMS patients were represented by RP patients.

Interestingly, the evidence of inflammatory activity between the first and second ALM courses was the unique variable associated with higher risk of MRI activity during OCR in the whole cohort. Similarly, the same variable was the only one associated with higher risk of relapse, MRI activity, and NEDA-3 status loss in the RRMS subgroup. Our findings are in line with those reported in core and extension phases of ALM trials[5,6,39] and real-world evidence.[40] Through the 4-year ALM extension study, a notable percentage of "early relapsers" (i.e. patients who relapsed between the first and second ALM course: 46% in CARE-MS I(6) and 60% in CARE-MS II(5)) received a third or subsequent courses of ALM. In the Italian study by Russo et al.,[40] 13.9% of patients experienced a relapse between the first two ALM courses, and this was linked to higher annualized relapse rate (ARR) during the remaining FU. On the contrary, in our study, the presence of inflammatory events (clinical and/or radiological) between the first two ALM courses seemed not to impact on the risk of disability worsening during OCR treatment. Although these findings seemed to be in line with 6-year FU data about ALM "early relapsers,"[39] we need a longer FU to confirm this statement in our population. In conclusion, we advise focusing the attention on patients who show signs of inflammatory activity between the first two ALM courses, suggesting that a de-escalation approach to low-efficacy DMTs as rescue strategy after ALM, may be hazardous.

Our study has several limitations, including the heterogeneity linked to its multi-center setting, the relatively short FU and the availability of blood samples only for patients' subgroups, also for the concomitance of Sars-Cov2 pandemic. Further prospective studies are needed to investigate whether the switch to OCR may be preferable to additional ALM re-treatments in patients who show persistence of disease activity after the first two ALM courses.

Conclusion

The switch to OCR after the first two ALM courses seemed to have a good safety and efficacy profile. We

did not observe significant differences in terms of infections with respect to clinical trials and available real-life data. Notably, a relevant proportion of patients developed early IgM hypogammaglobulinemia after OCR initiation, but longer FU is needed to assess its impact on infectious risk. Finally, OCR treatment seemed to play a relevant role in preventing ALM-related secondary autoimmune complications. A very good performance of OCR both in terms of relapses, MRI activity, and NEDA-3 achievement was observed in RRMS and PMS populations, even if an alert must be placed on patients who show inflammatory activity between the first two ALM courses.

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Data availability statement

Data are available upon reasonable request from the corresponding author.

Declaration of Conflicting Interests

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