Protocol Title: Evaluating the effects of short-term B-cell depletion on long-term disease activity and immune tolerance in relapsing multiple sclerosis.

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Contents

Synopsis:

Protocol title: Evaluating the effects of short-term B-cell depletion on long-term disease activity and immune tolerance in relapsing multiple sclerosis.

Indication: Relapsing multiple sclerosis (MS).

Objectives

- To determine whether two courses of treatment with ocrelizumab is associated with long-term suppression of disease activity in people with highly active relapsing MS.
- To test if two courses of treatment with ocrelizumab corrects the B-cell tolerance defects in patients with relapsing MS.

Study design: Open-label intervention.

Number of patients: 10

Target population: Adult patients with relapsing MS

Inclusion criteria:

- Age 18 years and older.

- Diagnosis of RRMS based on the latest revision of McDonald criteria

- At least two Gd-enhancing lesions on the brain or spinal cord MRI done in the prior three months

- Naïve to disease modifying therapies or using an injectable therapy (interferons or glatiramer acetate); or, if history of receiving natalizumab, fingolimod, dimethyl fumarate and teriflunomide, no exposure for past three months

- Expanded Disability Status Scale (EDSS) score at the time of screening =<3

Exclusion criteria:

- Contraindication to treatment with anti-CD20 antibodies, including being seropositive for HBsAg or HIV antibody or T-spot (or Quantiferon-Gold) positive
- Ever received B-cell depleting antibodies (rituximab, ocrelizumab, ofatumumab), alemtuzumab, daclizumab, mitoxantrone or hematopoietic stem-cell transplant
- Female who are pregnant, nursing or unwilling to use contraception up to six months after the second course of the infusion (or 12 months after the first infusion)
- Treatment with steroids in the past 30 days
- Clinically unstable medical or psychiatric disorders at the time of screening that require acute treatment as determined by the PI

Study duration: 2.5 years after the last dose of B-cell depleting antibody, ocrelizumab.

Description of study intervention: Participants will receive two courses of treatment with ocrelizumab, an FDA-approved medication for treatment of relapsing MS. The ocrelizumab infusions will not be

repeated after these two courses. Patient will be monitored for the return of clinical or MRI disease activity for at least 2.5 years after the last dose ocrelizumab. Immune cell phenotype and B-cell tolerance characteristics will be assessed before start of the treatment with ocrelizumab and 12-18 months after the second course of ocrelizumab infusion.

Assessments:

The primary endpoint (the failure event) of this study is the time to return of disease activity after the third month post-first-infusion, objectively demonstrated by development of new T2 hyperintense lesions or Gd-enhancing lesions on the MRI or a clinical relapse that is confirmed with an objective change in the neurological examination or a corresponding new or enhancing lesion on the MRI that is obtained after the onset of symptoms.

Mechanistic studies will be performed on the peripheral blood mononuclear cells obtained before the infusions and periodically after the infusions.

Study locations: This study will be conducted at Johns Hopkins University (JHU).

Sample size and statistical analysis

This is a pilot proof of concept study and no formal sample size calculations are performed.

We will recruit 10 patients in this study.

Introduction

Background and rational for performing this study

Disease modifying treatments and relapsing multiple sclerosis

Controlling and even stopping the inflammatory disease activity (i.e. clinical relapses and gadolinium enhancing or new T2 lesions on the MRI) is nowadays possible in most patients with relapsing forms of multiple sclerosis (MS). However, in most instances, it requires continuous and long-term treatment with a disease modifying treatment (DMT), which is costly, disruptive to the life of patients, associated with a sense of having a chronic disease and potentially adverse effects that are commonly seen with prolonged immunosuppressive therapies (opportunistic infections and cancers). To control the disease activity, almost all the available DMTs need to be used continuously. Stopping them is associated with return of the disease activity and in the case of two medications (fingolimod and natalizumab) with the possibility of more severe return of the disease activity compared to the pre-DMT baseline (rebound phenomenon).

There are currently two strategies that can result in medication-free prolonged freedom of disease activity in a proportion of patients: hematopoietic stem cell transplantation and treatment with alemtuzumab. Intense immunosuppression, followed by autologous bone marrow transplantation stopped the disease activity in more than 80% of patients with highly active relapsing MS. Two courses of treatment with anti-CD52 monoclonal antibody, alemtuzumab, have also been reported to be associated with prolonged freedom from clinical and radiological disease activity. In a report from 5 years of extension of the pivotal clinical trials of alemtuzumab in relapsing MS (CARE-MS studies), more than 60% of patients who only received two courses of alemtuzumab, did not need retreatment. However, both these approaches have high morbidity and are even associated with mortality, are reserved for cases with extremely severe disease and patients who have accumulated disability and their disease activity could not be controlled with other approaches.

In an ideal situation, a short course of treatment with a safe disease modifying therapy would have long-term or permanent effects on immune tolerance and stop the disease activity.

B-cell depleting antibodies and relapsing MS

B-cell depleting anti-CD20 monoclonal antibodies, rituximab and ocrelizumab, have been shown to reduce the disease activity and using the most objective measure (Gd-enhancing lesions on the MRI), they effectively stop the disease. The short-term use of these medications has been safe and aside from preventable or manageable infusion reactions, no short-term serious side effects have been associated with them. However, based on the current practice and the medication label, these antibodies are supposed to be infused either every six months or at intervals trying to keep the peripheral blood depleted of B-cells. Long-term B-cell depletion has known and predictable side effects (such as hypogammaglobulinemia) and possible unpredictable adverse effects (e.g. increased risk of infection or cancer). For example, in two pivotal clinical trials of ocrelizumab in RRMS, the rate of upper respiratory infection and nasopharyngitis was mildly elevated, compared to the interferon beta-1a. However, across the clinical trials (in both RRMS and PPMS), the rate of developing the first neoplasm seemed to be twice as high as the comparator groups (0.4 per 100 patient-years of exposure versus 0.2 per 100 patient-years of exposure to other agents, including placebo and interferon beta-1a). It is conceivable that longer duration of exposure to these medications may be associated with increased number of cases

experiencing serious side effects, including infections and perhaps cancer. Long-term use of these medications is also very costly. However, there is evidence that even short-term treatment with B-cell depleting agents can result in prolonged suppression of disease activity. For example, long term follow-up of patients who participated in a phase II clinical trial of ocrelizumab demonstrated that the relapse rate did not increase after the B-cell repopulation and only one patient in the ocrelizumab group had a Gd-enhancing lesion 18 months after the 4th and final ocrelizumab infusion, similar to the 12 month data following only one cycle of rituximab infusion in the phase two trial. Our colleagues have reported cessation of disease activity in four patients with neuromyelitis optica for a long period of time after they stopped treatment with rituximab. Based on these data and experiences, we believe that even a short course of treatment with anti-CD20, B-cell depleting antibodies can result in prolonged drug-treatment-free cessation of disease activity, at least in a subset of patients. With treatment with anti-CD20 therapies for a limited period (instead of long-term treatment), we probably avoid the side effects of prolonged lack of B-cells in the circulating blood. If a short course treatment with these medications has similar efficacy to continuous treatment, aside from lower probably of experiencing side effect, it puts much smaller financial burden on patients, their families and the health system. This strategy would put an end to the controversy of whether to start people with MS on a safe, but less efficacious medication (escalation approach) or on a potent, but with potential or unknown long-term side effects (induction approach). If temporary B-cell depletion stops the disease activity in a large proportion of patients, its safety and inexpensiveness allow us to use it almost universally in most people with MS. The next step would be trying to find biomarkers that might be associated with this long-term suppression of the disease activity.

Anecdotal and early phase clinical trials evidence of long-term efficacy of short-term B-cell depletion

The principal investigator (BN) has reviewed clinic charts of patients with highly active relapsing MS who were treated with rituximab and found four cases that stopped this medication after one or two courses without starting another DMT. There was no return of radiological disease activity even several years after cessation of treatment with rituximab (unpublished data).

The long-term efficacy data in a phase II trial of ocrelizumab in relapsing MS reported only one patient (among more than 180 patients with follow-up data) developed enhancing lesions on the MRI, 18 months after the last ocrelizumab infusion. These results demonstrate that reinfusion of anti-CD20 antibodies every six months is probably unnecessary in most MS patients.

B-cell tolerance defects in MS and other autoimmune diseases

Defects in early B-cell tolerance checkpoints are associated with many autoimmune diseases including MS, type 1 diabetes (T1D), rheumatoid arthritis (RA), pediatric systemic lupus erythematosus (SLE) and systemic sclerosis (SS), and result in large numbers of circulating autoreactive B-cells in the blood of these patients. Early B-cell tolerance checkpoints include a central B-cell selection step in the bone marrow (BM) that is vastly dependent on sensing self-antigen binding to B-cell receptors (BCRs) and most likely Toll-like receptors (TLR),

whereas the peripheral B-cell tolerance checkpoint appears to require functional regulatory T cells (Treg) to prevent the accumulation of autoreactive mature naïve B-cells. In line with these observations, polymorphisms in PTPN22, BLK, LYN, CSK, BANK1 identified by GWAS studies as susceptibility genes for RA, SLE, or T1D but not MS, encode components regulating BCR signaling and likely result in the impaired central B-cell tolerance characteristic of T1D, RA, and SLE. In contrast, people with MS do not display gene

polymorphisms linked to the BCR signaling pathway and most of these patients establish normal central B-cell tolerance. However, people with MS suffer from an impaired peripheral B-cell tolerance checkpoint, resulting in the accumulation of autoreactive mature naïve B-cells in their blood potentially linked to alterations of their Treg function and IFN-gamma and IL-17 production. Interestingly, IL-17 production by T cells and T follicular helper (Tfh) development is favored by IL-6, a pro-inflammatory cytokine that is secreted with GM-CSF by B-cells from people with MS. Since anti-B-cell therapy normalizes IL-6 production in newly generated B-cells, this regiment may therefore also decrease IL-17 production by T cells including Tregs and circulating Tfh in treated people with MS in remission. In addition, B-cells have been shown to support the maintenance of memory T cells and their elimination may therefore favor the elimination of self-antigen specific T cells secreting inflammatory cytokines. Moreover, transitional B-cells enhanced after anti-B-cell therapy may also secrete increased amount of IFN-alpha, which has been shown to improve the condition of people with MS. We hypothesize that long-term suppression of disease activity in MS is the result of anti-B-cell therapy eliminating autoreactive B-cells in patients' blood and that newly generated B-cells after B-cell repopulation will not contain autoreactive clones associated with autoimmunity, an observation that may correlate with a normalized Treg compartment that no longer secrete inflammatory cytokines. Hence, a short two course of B-cell depletion may correct the peripheral B-cell tolerance defect seen in most people with relapsing MS and this normalization may be responsible for the long-term suppression of disease activity.

Specific aims and study overview

Aim1: To determine whether two courses of treatment with ocrelizumab is associated with long-term suppression of disease activity in people with highly active early relapsing MS.

We hypothesize that only two courses of treatment with ocrelizumab in subjects with early relapsing MS will stop the disease activity, as measured by new T2 or Gd-enhancing lesions on brain and spinal cord MRI, even long after B-cell repopulation in the blood. In an open label unblinded trial, we will enroll 10 patients with relapsing MS and with at least two Gd-enhancing lesions on the brain and spinal cord MRI and treat them with two courses of ocrelizumab and follow them clinically and radiologically for at least two and a half years.

Aim 2: **To test if two courses of treatment with ocrelizumab corrects the B-cell tolerance defects in patients with relapsing MS.** We hypothesize that B-cells repopulating after a two course-treatment with ocrelizumab will NOT contain an elevated proportion of autoreactive clones observed pre-treatment. In the patients recruited in aim 1, we will harvest pre-treatment and post-repopulation peripheral B-cells and assess early B-cell tolerance checkpoints by testing the reactivity of recombinant antibodies cloned from single B-cells.

Risk/benefit assessment

Known potential benefits

With stopping ocrelizumab after two courses, participants will be less likely to suffer from immunosuppressive effects of this medication. The long-term safety of this medication is also not known and with limiting the exposure of participants to only two courses of treatment, they will not be exposed to unknown risk associated with long term use of this medication.

Known Potential Risks

By limiting the participants to two courses of treatment with ocrelizumab, participants are at higher risk of return of MRI or clinical disease activity. However, it is well within the standard practice to start patients with safer, but less efficacious disease modifying treatments and if the disease activity continued, to escalate to higher efficacy medications. Even in the case of more effective medications such as alemtuzumab, the standard practice is treatment with only two courses of the medication and retreatment if the disease activity returns. In this study, we will restart the treatment with ocrelizumab in patients who experience the return of the disease activity, a practice similar to escalation or retreatment which are standards of care.

Loss of privacy is another risk of participating in this study. Several mechanisms will help prevent loss of privacy. Access to the data will be limited to study personnel and the database will be password-protected. Furthermore, each member of the study team will be limited to only the data they need to perform their tasks so that privacy is protected as much as possible. Only study personnel who need to access the database will be given such access.

The risks associated with the study are minimal, and an adequate protection plan is in place; as such, the potential benefits exceed the potential risks. The participation of patients is completely voluntary; the alternative to the patients is to not participate in the study. Lack of participation will in no way compromise the relationship they have with their neurologists or the ongoing care they receive for their MS.

Study objectives

Primary objective

To determine whether two courses of treatment with ocrelizumab is associated with long-term suppression of disease activity in people with highly active, relapsing MS.

Secondary objective

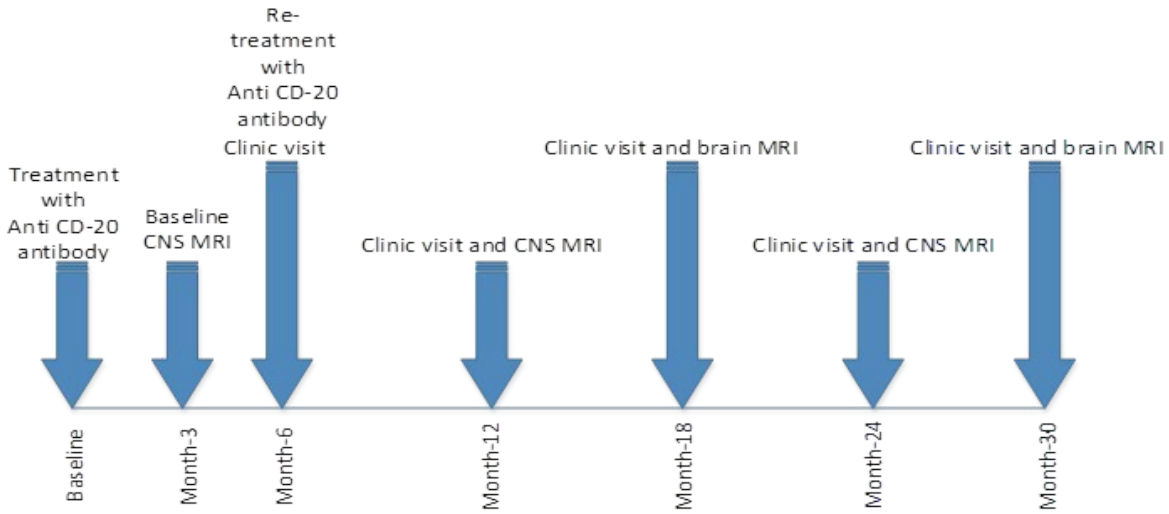
To determine whether these two courses of treatment with ocrelizumab correct the B-cell tolerance defects in patients with relapsing MS.

Investigational plan

Study design

This is an open label, single-arm study of anti-CD20 antibody, ocrelizumab in people with relapsing MS. Patients who fulfill the eligibility criteria will undergo blood draw for collection of peripheral blood mononuclear cells (PBMC). Participants will receive two infusions of ocrelizumab (300 mg) two weeks apart and then six months later (one infusion of 600 mg of ocrelizumab), according to the medication label and the standard of care. Baseline brain, cervical and thoracic MRI will be obtained about 12 weeks after the first course of treatment. Participants will be followed with clinical visits at least twice a year (or more frequently, if needed) and brain MRI (every six months) and cervical and thoracic spine MRI, at least annually, up to two and a half years after the baseline MRI.

The trial design is depicted in the following diagram:



Rationale of study design

The reason we chose a single arm, open label study, as opposed to a blinded controlled study is because the latter does not help with answering our research question. Our research question is if two courses of treatment with anti-CD20 antibodies have long lasting effects on the suppression of the disease activity. One thought regarding a controlled trial design would be a non-inferiority trial with the control group continuing the use of anti CD20 treatment (every 6 months), similar to the current practice. However, as we mentioned above, continued use of B-cell depleting antibodies is extremely capable in suppressing the disease activity and in our opinion, there is no equipoise about the ability of continued B-cell depletion in completely suppressing the disease activity. However, as we argued earlier, continued use of these antibodies have known and unknown long-term side effects and a three-year trial will not be able to demonstrate the long-term safety. Also, the cost of continuous treatment is not comparable to the treatment with only two courses of antibody infusion.

End of Study Definition

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the diagram above.

Study Population

Participants will be recruited at the Johns Hopkins MS Center. We will recruit about 10 patients with relapsing MS who are naïve to DMTs or have been on injectable DMTs (interferons or glatiramer acetate) or have been off of natalizumab, fingolimod or dimethyl fumarate for at least three months and have at least 2 Gd-enhancing lesions on the brain and/or spinal cord MRI on a scan obtained in three months prior to enrollment.

Inclusion criteria:

- Age 18 years and older.
- Diagnosis of RRMS based on revised McDonald criteria
- At least two Gd-enhancing lesions on the brain or spinal cord MRI done in the prior three months
- Naïve to disease modifying therapies or using an injectable therapy (interferons or glatiramer acetate); or, if history of receiving natalizumab, fingolimod, dimethyl fumarate and teriflunomide, no exposure for past three months
- Expanded Disability Status Scale (EDSS) score at the time of screening =<3

Exclusion criteria:

- Contraindication to treatment with anti-CD20 antibodies, including being seropositive for HBsAg or HIV antibody or T-spot (or Quantiferon-Gold) positive
- Ever received B-cell depleting antibodies (rituximab, ocrelizumab, ofatumumab), alemtuzumab, daclizumab, mitoxantrone or hematopoietic stem-cell transplant
- Female who are pregnant, nursing or unwilling to use contraception up to six months after the second course of the infusion (or 12 months after the first infusion)
- Treatment with steroids in the past 30 days
- Clinically unstable medical or psychiatric disorders at the time of screening that require acute treatment as determined by the PI

SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but do not subsequently receive the study intervention or enter in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Intervention

Investigational treatments

The intervention in this study is the two courses of infusions of ocrelizumab, an FDA approved medication for relapsing MS. The patients will receive ocrelizumab 300 mg IV infusion on days one and 15, according to the standard infusion protocols. The protocols include pre-medicating with acetaminophen 650 mg orally, diphenhydramine 50 mg orally and methylprednisolone 125 mg IV. The anti-CD20 antibody infusion will be repeated in six months (single infusion of ocrelizumab 600 mg). These infusions are standard of care and are done according to the medication label. However, the infusions will not be repeated after these two courses of infusion (the first two infusion of 300 mg ocrelizumab two weeks apart is considered one course and the infusion of 600 mg of ocrelizumab six months later is considered the second course).

Treatment arms

This is an open label, single arm, unblinded interventional study.

Treatment assignment

Patients who meet eligibility criteria are offered to participate in the study. This is an open label, unblinded interventional study, in which all participants receive essentially the same intervention.

Study intervention discontinuation and participant discontinuation/withdrawal

Discontinuation of study intervention

The study intervention is treatment with two courses of ocrelizumab. So, discontinuation from study intervention equates to not completing two courses of treatment with ocrelizumab or restarting the ocrelizumab or starting another MS disease modifying treatment after two courses of treatment with ocrelizumab for reasons other than breakthrough disease activity. All patients started on treatment will be followed in the study. Participants are free to withdraw from participation in the study at any time upon request. If a participant is unable to obtain insurance authorization for ocrelizumab within 90 days of screening visit (absent an administrative delay or other reason that is expected to be easily resolvable), the participant will be deemed a screen failure and will be replaced.

Lost to follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 consecutive scheduled visits and is unable to be contacted by the study staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The study staff will attempt to contact the participant and reschedule the missed visit within 1 month of the missed visits, counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study. Attempts to reschedule the missed visit will continue up to 3 months after the target visit date. Beyond 3 months, the visit will be considered missed and the visit window for the next standard of care clinic visit will open.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and at least 2 attempts to reach each of their emergency contacts and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable (3 consecutive visits missed and no response to repeated attempts to contact), he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

Schedule of events

Study visits will be conducted at the Johns Hopkins Outpatient Center (JHOC). Aside from obtaining informed consent for participation in the study and collecting peripheral blood mononuclear cells (PBMCs) and the NeuroQoL, all other procedures are part of the routine clinical and standard of care at Johns Hopkins MS Center. The only deviation from the standard of care in this study is stopping the ocrelizumab infusions after two courses. Study procedures are depicted in the following table (procedures in bold font are standard of care):

Tests and assessments	Screening visit	Infusion visits (month 0)	Month 3 (baseline)	Month 6	Month 12	Month 18	Month 24	Month 30
Informed Consent	X							
Verifying eligibility	X							

Medical history	X		X	x	x	x	x	x
Physical examination and vital signs assessment	X		X	x	x	x	x	x
Neurological examination (EDSS)	X		X	x	x	x	x	x
Blood work to assess eligibility for receiving ocrelizumab and its safety after the infusions	X		X	X	X			
OCT	X		X		X		X	
Brain MRI			x		x	x	x	x
Cervical/thoracic MRI			x		x		x	
NeuroQoL	X		X	x	x	x	x	x
Lymphocyte subset analysis			X	X	X	X		
Blood sample collection for storage and future analyses	X		X	X	X	X		
Blood draw and collection to be sent to Yale	X					X (or months 24)		
Ocrelizumab infusions		X 2 infusions two weeks apart		X 1 infusion				
Side effects assessment			X	x	x	x	x	x

Changes in the study visits and procedures due to response to the CIVD-19 pandemic:

Considering the recent events (March 2020) surrounding the COVID-19 pandemic and JHU response, and the fact that our study participants can be particularly vulnerable to the infection with the novel coronavirus, we will implement the following changes in our study protocol to reduce the immediate hazard to our participants related to the risk of exposure to COVID-19:

- Follow-up visits, including Month 3, Month 6, Month 12 Month 18, Month 24 and Month 30 in-person visits can be replaced with telemedicine visits with the study PI.
- The medical history, symptom evaluation and side effects assessment components of the study visits will be conducted through the telemedicine visits.
- The physical examination, vital signs assessment, neurological examination, OCT and research blood sample collection for future analysis will be paused temporarily, as they would require an in-person visit and would pose a risk to the participants and the staff. They will be resumed as soon as possible, after the resumption of normal research activities at the JHU.
- The NeuroQoL quality of life assessment will be delivered to particpants electronically. Secure Redcap surveys will be sent to the participants' email address. Results will be printed and filed in the study binders as usual.

- The blood work to assess and monitor safety and adverse effects of the therapy, including lymphocyte subset analysis, will continue to be ordered and results will be reviewed electronically by the PI.
- Infusions of ocrelizumab: Ocrelizumab may theoretically increase the risk of infection with the novel coronavirus. Delaying the ocrelizumab infusions will be determined on a case-by-case basis by the PI.
- MRI Procedures: MRI studies may be delayed because of the delays in the ocrelizumab infusions.

Screening visit: PI or designee will explain the study consent to study participants, and the study visit assessments will occur after the study participants sign the consent form. Screening visit procedures include review of eligibility criteria, physical exam, neurological examination, collecting vitals, blood work for assessing eligibility (liver function tests, complete blood count, Hepatitis- B surface antigen, Hepatitis-B core antibody, HIV antibody, lymphocyte subset including $CD19^{+}$ B-cells, total IgG and IgM and urine pregnancy test) and blood draw (for collecting PBMCs), and completion of Neuro-QoL adult item banks. Study participants will be enrolled into the study after the study physician confirms participants' eligibility to move forward with the study.

Obtaining the B-cell depleting antibody (ocrelizumab) and all the subsequent study visits and MRIs are part of routine and standard clinical care. If the B-cell depleting medication is not approved by the participants' insurance policy, the patient will be excluded from the study.

MRI procedures: MRI scans will be performed at one of the Hopkins Radiology facilities at 3T. Conventional T2/FLAIR/T1-weighted images as well as post-contrast T1-weighted images of the brain will be obtained at months 3 (baseline), 12, 18, 24 and 30. Conventional T2/STIR/T1-weighted images as well as post-contrast T1-weighted images of the cervical and thoracic spinal cord will be obtained at months 3 (baseline), 12 and 24. Similar to the standard of care, brain, cervical or thoracic spine MRIs may be done as needed if the patient develop symptoms that could be suggestive for an MS relapse (based on the evaluation by a study neurologist). The MRI scans will be read by an attending radiologist at Johns Hopkins University, and these readings will be used for making decisions regarding the presence of new T2 or Gd-enhancing lesions suggestive for re-emergence of the radiological disease activity.

Outcomes:

Primary outcome: The primary endpoint (the failure event) of this study is the time to return of disease activity after the third month post-first-infusion, objectively demonstrated by development of new T2 hyperintense lesions or Gd-enhancing lesions on the MRI or a clinical relapse. A relapse is defined as new or worsening neurologic symptom(s) with an objective change on the EDSS of at least 1.5 points for participants with baseline EDSS scores of 0 or 0.5 and at least 1-point change for participants with EDSS of 1 or more, as determined by the *examining neurologist*. Symptoms must have been attributable to MS, last ≥48 hours, been present at normal body temperature, and preceded by at least 30 days of clinical stability. If a new or worsening neurological symptom did not fulfil the above-mentioned criteria, but was associated with a corresponding new or enhancing lesion on the MRI that is done in 30 days after the start of that symptom, it is still considered a clinical relapse.

Secondary outcome:

Disability: six-month confirmed worsening or improvement of disability: We will report the proportion of patients who experience one step worsening or improvement of EDSS that is confirmed on a clinical visit six months later.

Quality of life measures: We will administer Neuro-QoL in the computer-adaptive test (CAT) format before the infusions and in all the subsequent clinic visits.

Assessment of T and B-cell phenotypes and function at baseline and 18-24 months post-B-cell depletion

The assessment T and B-cell phenotypes and function and analysis of the central and peripheral B-cell tolerance checkpoints, as well as measurement of cytokines will be done at Yale University. 30 milliliters of blood will be drawn from the participants before start of the treatment with ocrelizumab (at the screening visit) and 12 or 18 months after the second course of ocrelizumab infusion (month 18 or 24 of the study) and will be sent to Yale University for the analyses explained below.

We will investigate the T and B-cell phenotypes in the blood samples from the participants in the study, pre-treatment and after they repopulate their peripheral B-cells likely at either 18- or 24-months time points. We will follow the frequencies of $CD3^+CD4^+CD25^{hi}CD127^{lo}FOXP3^+$ Tregs, circulating $CD3^+CD4^+CXCR5^+PD\text{-}1^+$ Tfh cells increased in several autoimmune diseases, and other CD4+ and CD8+ naïve and memory T cell subpopulations. We will also assess the production of several cytokines including IL-2, IL-4, IL-6, IL_10, IL-17, Il-21, IFN-gamma and TNF-alpha by Tregs and other T cell subsets after activating PBMCs with phorbol-12-myristate-13-acetate (PMA) and ionomycin for 4 hours in the presence of GolgiStop (BD Biosciences) and intracellular staining with specific monoclonal anti-cytokine antibodies as previously reported by our laboratory. Hence, we will determine if the abnormal production of IL-17 and IFN-gamma previously identified in Tregs from people with MS or their elevated Tfh frequencies are corrected after two courses of anti-B-cell deletion. In addition, we will assess *in vitro* the suppressive function of Tregs from people with MS before and after B-cell deletion. Indeed, Tregs from people with MS have been reported to display decreased suppressive function and we will therefore be able to determine if ocrelizumab can normalize Treg function and if this feature is associated with long-term disease suppression.

We will also characterize various B-cell subpopulations in the blood of people with MS before and after treatment with ocrelizumab. We will follow the frequencies of $CD19^+CD10^+CD27^-IgM^{hi}CD21^{lo}$ transitional B-cells that recently emigrated from the bone marrow, $CD19^+CD10^-CD27^-IgM^+CD21^+$ mature naïve B-cells, $CD19^+CD10^-CD27^+IgM^+CD21^+$ circulating marginal zone/ IgM memory B-cells, $CD19^+CD10^-CD27^+IgM^-IgG^+CD21^+$ and $CD19^+CD10^-CD27^+IgM^-IgA^+CD21^+$ conventional IgG+ and IgA+ isotype switched B-cells as well as unconventional $CD19^+CD10^-CD27^-CD21^{lo}$ $CD11c^+$ memory B-cells that express T-bet, contain autoreactive clones and are generated after TLR activation in the presence of IFN-gamma and IL-21. The impact of anti-B-cell therapy on B-cell activation will also be determined by following CD69 expression that we previously reported to be increased on B-cells from people with MS. The expression of other B-cell activation markers such as CD80, CD86, TACI and FAS will also be assessed by flow cytometry on each

B-cell subsets described above. We will also obtain a preview on the peripheral B-cell selection in MS by characterizing their proliferative homeostasis history by following the expression of proliferation marker Ki67 and using the detection of Kappa recombination excision circles (KREC) by quantitative PCR in freshly isolated B-cell subpopulations from healthy controls and people with MS before and after anti-B-cell deletion as previously described. Indeed, we previously reported that mature naïve B-cells from people with MS have undergone increased homeostatic expansion in the periphery that may result from the amplification of autoreactive clones. Altogether, these studies will determine the impact of anti-B-cell therapy on the phenotype and function of T and B-cells in people with MS.

Analysis of the central and peripheral B-cell tolerance checkpoints

Single $CD19^{+}CD10^{hi}CD21^{-/lo}IgM^{hi}CD27^{-}$ new emigrant/transitional and $CD19^{+}CD10^{-}CD21^{+}IgM^{+}CD27^{-}$ mature naive B-cells will be isolated from the blood of these patients before and after treatment and frequencies of polyreactive, HEp-2 reactive and anti-nuclear clones will be determined as previously described. In brief, Ig heavy and light chain genes will be amplified by RT-PCR and 20-30 immunoglobulin genes from single new emigrant/transitional and mature naïve B-cells will be cloned and expressed in vitro. Recombinant antibodies will be tested for HEp-2 reactivity and polyreactivity by ELISAs and anti-nuclear reactivity using indirect fluorescence assay on HEp-2 slides as previously described. Recombinant antibodies will be considered polyreactive when they recognized all three antigens tested individually (dsDNA, insulin, and LPS) using supernatants tested at 1µg/ml antibody concentrations and three 1:4 dilutions in PBS as reported. In HEp-2 and polyreactivity ELISAs, ED38 recombinant antibody will be used as positive control. Hence, we will determine if B-cell depletion can correct the impaired removal of developing autoreactive B-cells in the periphery of people with MS and is associated with disease suppression.

Safety and adverse effect monitoring:

Potential participants will have lab work done (liver function tests, complete blood count, Hepatitis- B surface antigen, Hepatitis-B core antibody, HIV antibody, lymphocyte subset including $CD19^{+}$ B-cells, total IgG and IgM and urine pregnancy test) as part of routine clinical care to assess for study eligibility and as the baseline for future comparison. Patients who are positive for Hepatitis-B surface antigen (HBsAg) or Hepatitis-B core antibody (HBcAb) will be excluded from participation.

Ocrelizumab has been extensively used in people with MS. In this small study, with limited and short-term use of B-cell depleting antibodies, we do not expect to see unanticipated side effects worth reporting, however, the most important safety consideration in this setting is the return of disease activity after B-cell reconstitution. Unlike patients who stop medications such as natalizumab and fingolimod and may experience severe return of the disease activity, rebound phenomenon has never been described in association with cell depleting antibodies such as alemtuzumab, rituximab or ocrelizumab. For example, most patients who receive alemtuzumab have severe MS, uncontrolled with other DMTs. Most patients only receive two courses of treatment with alemtuzumab and after lymphocyte reconstitution, there has been no reports of severe return of disease activity (rebound). Similar to this situation, we do not anticipate that patients with B-cell reconstitution after B-cell depletion therapy will experience rebound. Although a subgroup of patients may have return of disease activity, the disease activity will be mild and non-disabling.

To comply with rules and regulations of performing clinical studies, we will record all safety data during the study. Upon signing the informed consent form, each participant will be given the names and telephone numbers of study staff for reporting adverse events and medical emergencies.

Adverse event (AE): An AE is the development or worsening of any undesirable symptom, sign or medical condition occurring after starting the study drug even if the event is not considered related to study medication. Medical conditions or diseases present before starting study drug are only considered AEs if they worsen after initiating study medication. At each study encounter, the occurrence of AEs should be sought by non-directive and directive questioning. AEs may also be collected when they are volunteered by the subjects during or between encounters.

Serious adverse event (SAE): An SAE is defined as an event which:

- is fatal or life-threatening

- results in persistent or significant disability

- constitutes a birth defect/congenital abnormality

- requires inpatient hospitalization for at least 24 hours or prolongation of existing hospitalization for at least 24 hours

- is medically significant

Data safety monitoring committee (DSMB): DSMB is not required for a small study using a medication that is FDA-approved for the condition, however, we will have the following treatment stop and study termination rules:

Treatment stop rules: Stopping the study is equivalent to restarting the B-cell depleting antibody (i.e. ocrelizumab) or another DMT, if there is medical contraindication to the continued use of ocrelizumab (e.g. development of allergic or severe infusion reactions during the last infusion). The followings are the criteria for restarting the DMT (stopping the study).

1. A clinical relapse, affecting optic nerves (with worst visual acuity of 20/50 or less) or causing motor weakness, ataxia, bladder or bowel symptoms. Pseudo-relapses are not uncommon among patients with MS. When in doubt, the PI will use MRI (similar to the standard of care) to confirm development of new lesions corresponding to patient's symptoms (based on the possible localization of the symptoms).

2. Development of two new T2 hyperintense lesions or two gadolinium enhancing lesions on any MRI after the baseline MRI. The baseline MRI was defined the MRI that is obtained three months after the first ocrelizumab infusion.

Data management

We will use REDCap (Research Electronic Data Capture) [https://projectredcap.org/], a secure web application to collect data, create the study database and access the data for analysis. Study coordinator will enter the data required by the protocol into the Electronic Case Report Forms (CRFs). The PI will assure that the data entered into CRFs are complete and accurate.

Sample size

We selected to recruit 10 participants in this open-label, non-controlled study. We limited the number of participants for two reasons: The pre- and post-infusion analysis of B-cell tolerance is expensive and time-consuming, and we could accommodate the analysis of only 20 samples (10 pre and 10 post-infusions samples) during the proposed time of the study. We also wanted to make sure that we would observe a prolonged drug-free absence of disease activity in a subset of participants, before recruiting a larger group of patients for a potential larger study.

The sample size is supported by our previous studies of central and peripheral B-cell checkpoints in patients with autoimmune diseases and healthy control subjects in which we had found that the frequency of autoreactive mature naïve B-cells was 48.9±3.06% in patients and 20.1±0.9% in healthy donors. Assuming that the frequency of autoreactive cells among post-treatment repopulated B-cells will be similar to normal controls, our proposed sample size of 10 subjects per group before and after treatment would give us more than 90% power to detect a significant difference with an α of 0.05 with a 2-sided Student's t-test. The analysis of each individual is very labor intensive and costly but fortunately, significant findings on the establishment of B-cell tolerance can be obtained with a relatively small number of subjects.

Statistical analysis

We will describe the demographic, clinical characteristics and MRI data of all participants before treatment with the B-cell depleting agent. We will report the proportion of participants who experience the primary endpoint of the study (return of the disease activity) and the time to failure event, using a Kaplan-Meyer curve. Proportion of patients with clinical relapse, new MRI activity or disability progression will separately be reported. Quality of life data will be analyzed using linear mixed effect models.

To look at the association between long-term response to B-cell depletion therapy and autoreactivity of B-cells at different stages of development, we will use logistic regression and Cox proportional hazards models with failure or time-to-failure event as the outcome variable and the above-mentioned proportion of auto-reactive B-cells at different stages of development and the change in proportions before and after treatment as the predictor variables. Failure event as defined in the Aim 1 is the return of disease activity after the third month post-first-infusion, objectively demonstrated by development of new T2 hyperintense lesions or Gd-enhancing lesions on the MRI or a clinical relapse that is confirmed with an objective change in the neurological examination or a corresponding new or enhancing lesion on the MRI that is obtained after the onset of symptoms.

Using paired t-test, we will compare the frequency of autoreactive mature naïve B-cells, Treg and Tfh cells and cytokine production before treatment with ocrelizumab and after the B-cell repopulation.

Ethical considerations

Regulatory and ethical compliance

The Investigators will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" which affords the greater protection to the individual. It is the mission of the physician to safeguard the health of the people. The study physicians' knowledge and conscience are dedicated to the fulfilment of this mission. The clinical study will fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guidelines for greater protection to the subject.

The study will be submitted to JHU IRB for approval prior to enrolling study participants into the study. The IRB will be notified annually about study progress, and all important updates on the study or medically important events associated with the study will be submitted to IRB as the study progresses.

The investigator will establish secure safeguards of confidentiality of research data as described in the current revision of the International Ethical Guidelines for Biomedical Research involving Human Subjects. The Health Insurance Portability and Accountability Act (HIPAA), also known as "The Privacy Rule", has set new standards and regulations to protect patients from inappropriate disclosures of their "protected health information" (PHI) that could cause harm to their insurability, employability and/or their privacy. PHI pertains to any information that can be used to identify an individual which is created, used, or disclosed in the course of providing a health care service, such as diagnosis or treatment. HIPAA does allow for researchers to access and use PHI when necessary to conduct research. The study records will be identified using subject's study number to protect the privacy of the study participants. The study team will follow the JHU IRB recommendations to protect the privacy of study participants. Study consent will also include the privacy language mandated by the Institution's IRB. Vulnerable populations (including fetuses, neonates, pregnant women, children and prisoners) will not be involved in this study. All members of the research teams at JHU have received required training in protection of human subjects in research and will receive refresher courses at intervals based on state and federal policies.

Informed consent procedures

The study investigators, or a person designated by the investigators will explain the IRB approved study consent to study participants and obtain signed informed consent from each subject. The study participants will be given ample time to review the consent, and are encouraged to ask questions during the consent process. After the subject and representative, have orally consented to participation in the trial, the witness' signature on the form will attest that the information in the consent form was accurately explained and understood. The study participants are encouraged to ask questions about the study during the study participation period. The investigator or designee must also explain to the subjects that they are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The study participants will be notified about the new study information during the study participation period, and will be re-consented when applicable. A copy of the signed informed consent form will be given to the subjects for their records.

Publication of the study protocol and results

The key design elements of this protocol will be posted in a publicly accessible database such as clinicaltrials.gov.

Protocol adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations. Any change or addition to the protocol can only be made in a written protocol amendment. Only amendments that are required for patient safety may be implemented prior to IRB approval.