STUDY TITLE:	Evaluating the effects of ocrelizumab on B-cell tolerance defect in relapsing multiple sclerosis
study drug:	OCREVUS (ocrelizumab)
SUPPORT PROVIDED BY:	Genentech, Inc.
INVESTIGATOR:	{Bardia Nourbakhsh0, 600, N Wolfe Street, Path 627, Baltimore, MD, 21287, Telephone: 410-614-1522, Fax: 410-502-6736, bnourba1@jhmi.edu}
Co-Investigators:	Abdel-Rahim A. Hamad, M.V.Sc., Ph.D.
	NCT04261790
Protocol version/date:	1. June, 18, 2020

Contents

INTRODUCTION

B-cell depleting anti-CD20 monoclonal antibody, ocrelizumab, has been shown to reduce the multiple sclerosis (MS) disease activity and using the most objective measure (Gd-enhancing lesions on the MRI), it effectively stops the disease. The short-term use of this medication has been safe and aside from preventable or manageable infusion reactions, no short-term serious side effects have been associated with them. However, it is not clear how depleting the peripheral blood from B-lymphocytes results in cessation of the disease activity. Here, we hypothesize that peripheral B-cell depletion corrects the B-cell tolerance defect that is seen in the majority of patients with MS. 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 (Figure 1).

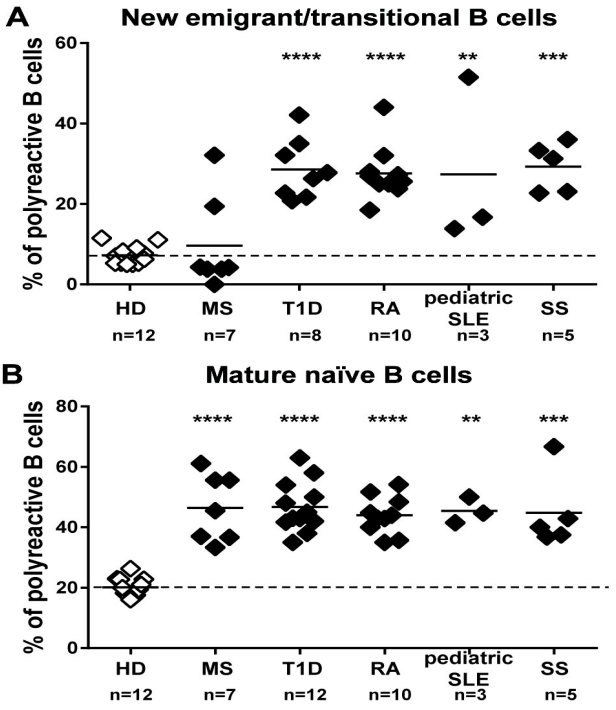

Figure 1. Normal central but defective peripheral B-cell tolerance checkpoint in MS. (**A**) Central B-cell tolerance is functional in most people with MS. The frequencies of polyreactive new emigrant/transitional B-cells that recently exited the bone marrow are elevated in patients with type 1 diabetes (T1D), rheumatoid arthritis (RA), pediatric systemic lupus erythematosus (SLE) and primary sjögren's syndrome (SS) but low in healthy donors (HD) and most people with MS. (**B**) All patients with autoimmune diseases display impaired peripheral B-cell tolerance checkpoint as illustrated by elevated proportions of mature naïve B-cells expressing antibodies that bind antigens from HEp-2 cell lysate compared to HD controls. The number of patients is indicated and stars indicate significant differences with HD (** P<0.01, *** P<0.001, **** P<0.0001).

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 genome-wide association studies (GWAS) 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 (Figure 1A) . 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 (Figure 1A). 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 and IL-17 production (Figure 1B). 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.[32] Moreover, transitional B-cells enhanced after anti-B-cell therapy may also secrete increased amount of IFN, 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, two courses 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.

OBJECTIVES

Primary Objectives

To test if two courses of treatment with a B-cell depleting antibody, ocrelizumab, corrects the B-cell tolerance defects in patients with relapsing MS.

STUDY DESIGN

DESCRIPTION OF THE STUDY

This is an open label, single-arm study of 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). Baseline MRI will be obtained 3 months 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, cervical and thoracic spine MRI, up to two and a half years after the baseline MRI.

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 3	Month 6	Month 12	Month 18	Month 24	Month 30

		(month 0)	(baseline)					
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 Dr.	X					X (or months 24)		

Hamad's lab at Hopkins								
Ocrelizumab infusions		X 2 infusions two weeks apart		X 1 infusion				
Side effects assessment			X	x	x	x	x	x

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, EDSS, collecting vitals, 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.

RATIONALE FOR 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 antibody, ocrelizumab corrects the B-cell tolerance defect seen in patients with relapsing-remitting multiple sclerosis.

OUTCOME MEASURES

PRIMARY OUTCOME MEASURE

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 Dr. Hamad's Lab at Johns Hopkins University. 50 milliliters of blood will be drawn from 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 Dr. Hamad's Lab for the analyses explained below. We will investigate the T and B-cell phenotypes in fresh blood samples from 10 individuals with MS 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+CD25hiCD127loFOXP3+ Tregs, circulating CD3+CD4+CXCR5+PD-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-10, IL-17, and IFN-gamma 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 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.

Because of the lack of lineage-specific markers or transcription factors, IL-10 expression will be used as a major readout to determine Bregs. We will use the following two methods: 1) Direct detection of IL-10 on total PBMCs stimulated with PMA+ionomycin (with Golgi block) for 4-5 hours followed by cell surface CD19/CD24/CD38/CD27 (with CD19+CD24hiCD38hiCD27- enriched in Bregs). 2) Detection of IL-10 produced by B cells after in vitro B cell activation. In brief, 250K CD20+ enriched B cells using magnetic beads will be activated for 18-24hours in vitro with 1ug/mL CpG-B and 0.05 ug/mL recombinant CD40L. B cells will then be stimulated with PMA+ionomycin (with Golgi block) for 4-5 hours followed by cell surface CD19/CD24/CD38/CD10/CD27 (with CD19+CD24hiCD38hiCD10hiCD27- enriched in Bregs/transitional B cells) and intracellular staining with anti-IL-10. We may better detect the production of IL-10 with this in vitro B cell stimulation protocol.

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-IgMhiCD21lo 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-CD21lo 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 peripheral B-cell tolerance checkpoints in people with MS before and after anti-B-cell therapy: We propose to assess the peripheral B-cell tolerance checkpoints in ten people with MS before and after treatment with ocrelizumab. Single CD19+CD10hiCD21-/loIgMhiCD27- 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.43 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.

Anticipated results and potential pitfalls: We anticipate that B-cell depletion will correct the altered phenotype and function of T and B-cells from people with MS and normalize the production of pro-inflammatory cytokines in both T and B-cells. In addition, we expect that the majority of people with MS before the anti-CD20 treatment, will display a normal central B-cell tolerance with low proportions of autoreactive new emigrant/transitional B-cells similar to those in normal controls as previously reported (Figure 1A). In contrast, we anticipate that the peripheral B-cell tolerance checkpoint will be defective in MS before ocrelizumab treatment as previously reported (Figure 1B). However, we expect that mature naïve B-cells after B-cell repopulation 18-24 weeks post initial anti-CD20 treatment will show a pattern of antibody autoreactivity similar to normal controls. In other words, a short-course of treatment with an anti-CD20 antibody would correct the peripheral B-cell tolerance defect in most people with MS and this correction to be associated with long-term absence of disease activity. On the other hand, in the 3-4 people with MS who may present baseline central B-cell tolerance defect (30% in Kinnunen et al. and Figure 1A), the effects of transient B-cell depletion therapy may not be long-lasting. We may further test if a two-course treatment may induce long lasting remission through the sustained establishment of proper B cell tolerance at least in some MS patients by evaluating again their proportions of autoreactive B cells two years later (48 month time point). Although these studies are beyond the time frame of this project, the samples collected during the current proposed analysis will allow us to address this question.

Secondary Outcome Measures

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; proportion of patients who experience one step worsening or improvement of EDSS that is confirmed on a clinical visit six months later; Neuro-QoL in the computer-adaptive test (CAT) format before the infusions and in all the subsequent clinic visits.

SAFETY PLAN

Patients will be evaluated at each study visit for the duration of their participation in the study. The safety plan is described in detail later in the protocol.

COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in accordance with current U.S. Food and Drug Administration (FDA) Good Clinical Practices (GCPs), and local ethical and legal requirements.

MATERIALS AND METHODS

SUBJECTS

Subject Selection

Participants will be recruited at the Johns Hopkins MS Center. We will recruit 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 one Gd-enhancing lesions on the brain and/or spinal cord MRI on a scan obtained in three months prior to enrollment OR have at least one new T2/FLAIR lesion on the brain or spinal cord MRI done in three months prior to enrollment (compared to a previous MRI performed within 18 months of the most recent MRI).

We plan to enroll participants who have active disease, as the absence of disease activity in the follow-up period in these patients would be more meaningful.

Inclusion and exclusion Criteria

Inclusion criterion
Diagnosis of RRMS based on revised McDonald criteria
At least one Gd-enhancing lesions on the brain or spinal cord MRI done in the prior three months OR at least one new T2/FLAIR lesion on the brain or spinal cord MRI done in the prior three months (compared to a prior MRI performed within 18 months of the most recent MRI)
Age>=18
Naïve to DMT or at least off these DMTs (natalizumab, fingolimod, DMF) for three months or on an injectable DMT (interferons or glatiramer acetate)
Expanded Disability Status Scale (EDSS) score at the time of screening =<3
A negative urine or serum pregnancy test must be available for premenopausal women and for women <12 months after the onset of menopause, unless they have undergone surgical sterilization
Women of childbearing potential must agree to remain abstinent (refrain from heterosexual intercourse) or use one method of contraception with a failure rate of <1% per year or a barrier method supplemented with spermicide. Contraception must continue for the duration of study treatment and for at least 24 weeks after the last dose of study treatment[$$]

Exclusion criterion
Contraindication to treatment with anti-CD20 antibodies, including being seropositive for HBsAg
Active hepatitis B virus infection
Ever received B-cell depleting antibodies (rituximab, ocrelizumab, ofatumumab), alemtuzumab, daclizumab, mitoxantrone or hematopoietic stem-cell transplant
Pregnant or lactating women
Hypersensitivity to ocrelizumab
Treatment with steroids in the past 30 days

$$ A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause of other than menopause), and has not undergone surgical sterilization (removal of the ovaries and/or uterus)

Examples of contraceptive methods with a failure rate of <1% per year include bilateral tube ligation, male sterilization, established hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices.

The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence and withdrawal are not acceptable methods of contraception.

Examples of barrier methods supplemented with the use of spermicide include male or female condom, cap, diaphragm, or sponge.

METHOD OF TREATMENT ASSIGNMENT

This is an open label, single arm, unblinded interventional study. All participants will receive similar treatment and intervention.

STUDY INTERVENTIONS

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).

STUDY ASSESSMENTS

Signed, IRB approved informed consent will be obtained from patients prior to the pretreatment assessments. We will ensure that informed consent will be obtained for any mandated testing or screening process for this protocol.

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

MRI procedures: MRI scans will be performed as part of the standard of care. 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.

Assessments during Treatment

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 Dr. Hamad's lab at Hopkins	**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 in to response to the CIVD-19 pandemic:

Considering the recent events surrounding the COVID-19 pandemic and JHU response, and the fact that

our study participants can be theoretically 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: 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.

DISCONTINUATION OF PROTOCOL-SPECIFIED THERAPY

Protocol-specified therapy may be discontinued for any of the following reasons:

- Unacceptable toxicity
- Patient election to discontinue therapy (for any reason)
- Physician's judgment

SUBJECT DISCONTINUATION

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 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 after three months subsequent to the first ocrelizumab infusion.

Termination rule

If four patients during the study required retreatment with ocrelizumab (or another DMT), we will terminate the study (i.e. will offer all participants the choice to resume their DMT).

STUDY DISCONTINUATION

Genentech Study Center, and the Principal Investigator has the right to terminate this study at any time. Reasons for terminating the study may include the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory
- Data recording are inaccurate or incomplete
- Study protocol not followed

STATISTICAL METHODS

We will describe the demographic, clinical characteristics and MRI data of all participants before treatment with the B-cell depleting agent. Using paired t-tests, 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. We will report the proportion of participants who experience the 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 by 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.

Missing Data

Because of the small sample size and close follow-up, we do not expect to encounter missing data problem. Also, each participant will be his/her own control and no between-patient comparison will be made.

Determination of Sample Size

Patients' numbers are also based on 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.

Data Management

We will use Johns Hopkins 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.

Data Quality Assurance

Accurate, consistent, and reliable data will be ensured through the use of standard practices and procedures.

SAFETY REPORTING OF ADVERSE EVENTS

ASSESSMENT OF SAFETY

Specification of Safety Variables

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) per protocol. This includes all events of death, and any study specific issue of concern.

Adverse Events

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with multiple sclerosis that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations)
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

Serious Adverse Events

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the IMP.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

METHODS AND TIMING FOR ASSESSING AND RECORDING SAFETY VARIABLES

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study are collected and reported to appropriate IRB(s), and Genentech, Inc.

Adverse Event Reporting Period

The study period during which AEs and SAEs as described in section J where the patient has been exposed to Genentech product must be reported. Reporting period begins after informed consent is obtained and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the ocrelizumab (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes

There is a plausible temporal relationship between the onset of the AE and administration of the {study drug}, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the ocrelizumab or with similar treatments; and/or the AE abates or resolves upon discontinuation of the ocrelizumab or dose reduction and, if applicable, reappears upon re- challenge.

No

Evidence exists that the AE has an etiology other than the ocrelizumab (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to ocrelizumab administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert (P.I) or current Investigator Brochure (I.B).

Unexpected adverse events are those not listed in the P.I or current I.B or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

PROCEDURES FOR ELICITING, RECORDING, AND REPORTING ADVERSE EVENTS

Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation time points should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is acceptable to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section I), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study

e. Assessment of Severity of Adverse Events

The adverse event severity grading scale for the NCI CTCAE (v5.0 Update current versions) will be used for assessing adverse event severity. Below Table should be used for assessing severity for adverse events that are not specifically listed in the NCI CTCAE.

Adverse Event Severity Grading Scale for Events Not Specifically Listed in NCI CTCAE

Grade	Severity
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; or intervention not indicated
2	Moderate; minimal, local, or non-invasive intervention indicated; or limiting age-appropriate instrumental activities of daily living [a]
3	Severe or medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; or limiting self-care activities of daily living [b,c]
4	Life-threatening consequences or urgent intervention indicated d
5	Death related to adverse event [d]

NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

Note: Based on the most recent version of NCI CTCAE (v5.0), which can be found at:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

a. Instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

b. Examples of self-care activities of daily living include bathing, dressing and undressing, feeding oneself, using the toilet, and taking medications, as performed by patients who are not bedridden.

c. If an event is assessed as a "significant medical event," it must be reported as a serious adverse event

d. Grade 4 and 5 events must be reported as serious adverse events

f. Pregnancy

If a female subject becomes pregnant while receiving the study drug or within 6 months after the last dose of study drug, or if the female partner of a male study subject becomes pregnant while the study subject is receiving the study drug or within 6 months after the last dose of study drug, a report should be completed and expeditiously submitted to Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the study drug should be reported as an SAE.

Additional information on any ocrelizumab-exposed pregnancy and infant will be requested by Genentech/Roche Drug Safety at specific time points (i.e. after having received the initial report, at the end of the second trimester, 2 weeks after the expected date of delivery, and at 3, 6, and 12 months of the infant's life).

g. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior ocrelizumab exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE adequately to Genentech drug Safety during follow up period

h. Case Transmission Verification of Single Case Reports

The Sponsor agrees to conduct the Case Transmission verification to ensure that all single case reports have been adequately received by Genentech via the sponsor emailing Genentech a Quarterly line-listing documenting single case reports sent by the sponsor to Genentech in the preceding time period.

The periodic line-listing will be exchanged within seven (7) calendar days of the end of the agreed time period. Confirmation of receipt should be received within the time period mutually agreed upon.

If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution. The sponsor shall receive reconciliation guidance documents within the 'Activation Package'.

Following Case Transmission Verification, single case reports which have not been received by Genentech shall be forwarded by the sponsor to Genentech within five (5) calendar days from request by Genentech.

At the end of the study, a final cumulative Case Transmission Verification report will be sent to Genentech

i. AEs of Special Interest (AESIs)

AESIs are a subset of Events to Monitor (EtMs) of scientific and medical concern specific to the product, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor is required. Such an event might require further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the trial Sponsor to other parties (e.g., Regulatory Authorities) may also be warranted.

Adverse events of special interest for this study include the following:

- Cases of potential drug-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law:
    - Treatment-emergent ALT or AST □ 3 □ ULN in combination with total bilirubin □ 2 □ ULN
    - Treatment-emergent ALT or AST □ 3 □ ULN in combination with clinical jaundice
- Data related to a suspected transmission of an infectious agent by the study drug, as defined below:
    - Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

j. Exchange OF SINGLE CASE REPORTS

The sponsor will be responsible for collecting all protocol-defined Adverse Events (AEs)/Serious Adverse Events (SAEs), AEs of Special Interest (AESIs), Special Situation Reports (including pregnancy reports) and Product Complaints (with or without an AE) originating from the Study for the Product.

Investigators must report all the above mentioned single case reports adequately to Genentech within the timelines described below. The completed MedWatch or CIOMS I form or Genentech approved reporting forms should be faxed/emailed immediately upon completion to Genentech at the following contacts:

All protocol-defined AEs, SAEs, AESIs, Special Situation Reports (including pregnancy reports) and Product Complaints with an AE should be sent to:

Fax: 650-238-6067

Email: usds_aereporting-d@gene.com

All Product Complaints without an AE should be sent to:

Email: kaiseraugst.global_impcomplaint_management@roche.com

It is understood and agreed that the Sponsor will be responsible for the evaluation of AEs/SAEs, AESIs, Special Situation Reports (including pregnancy reports) and Product Complaints (with or without an AE) originating from the study.

These single case reports will be exchanged between the parties as outlined below so that regulatory obligations are met.

Serious adverse events (SAEs), pregnancy reports (including pregnancy occurring in the partner of a male study subject) AEs of special interest (AESIs) and Special Situation Reports, where the patient has been exposed to the Genentech Product, will be sent on a MedWatch form or CIOMS I form or on Genentech approved reporting forms to Genentech Drug Safety. Transmission of these reports (initial and follow-up) will be either electronically or by fax and within the timelines specified below:

- SADRs

Serious AE reports that are related to the Product shall be transmitted to Genentech within fifteen (15) calendar days of the awareness date.

- Other SAEs

Serious AE reports that are unrelated to the Product shall be transmitted to Genentech within thirty (30) calendar days of the awareness date.

- AESIs

AESIs shall be forwarded to Genentech within fifteen (15) calendar days of the awareness date.

- Special Situation Reports

  Pregnancy reports

While such reports are not serious AEs or Adverse drug Reactions (ADRs) per se, as defined herein, any reports of pregnancy (including pregnancy occurring in the partner of a male study subject), where the fetus may have been exposed to the Product, shall be transmitted to Genentech within thirty (30) calendar days of the awareness date. Pregnancies will be followed up until the outcome of the pregnancy is known, whenever possible, based upon due diligence taken to obtain the follow-up information.

- Other Special situation reports

In addition to all SAEs, pregnancy reports and AESIs, the following Special Situations Reports should be collected even in the absence of an Adverse Event and transmitted to Genentech within thirty (30) calendar days:

- Data related to the Product usage during pregnancy or breastfeeding
- Data related to overdose, abuse, off-label use, misuse, inadvertent/erroneous administration, medication error (including potentially exposed in case of medication errors or intercepted medication errors) or occupational exposure, with or without association with an AE/SAE unless otherwise specified in the protocol
- Lack of therapeutic efficacy
- Drug interaction
- Use of a Medicinal Product in a Pediatric and Elderly population (in addition, reasonable attempts should be made to obtain and submit the age or age group of the patient, in order to be able to identify potential safety signals specific to a particular population)
- Product Complaints

  All Product Complaints (with or without an AE) shall be forwarded to Genentech within fifteen (15) calendar days of the awareness date.

  A Product Complaint is defined as any written or oral information received from a complainant that alleges deficiencies related to identity, quality, safety, strength, purity, reliability, durability, effectiveness, or performance of a product after it has been released and distributed to the commercial market or clinical trial.

MEDWATCH 3500A REPORTING GUIDELINES

In addition to completing appropriate patient demographic (Section A) and suspect medication information (Section C & D, the report should include the following information within the Event Description (section B.5) of the MedWatch 3500A form:

- Protocol number and title description

- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics (Section B.6)
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-Up Information

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B. initial, patient number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

Reporting to Regulatory Authorities, Ethics Committees and Investigators

Genentech as the Marketing Authorization Holder will be responsible for the reporting of individual case safety reports from the study to the regulatory authority in compliance with applicable regulations.

The sponsor will be responsible for the expedited reporting of safety reports originating from the Study to the EMA through Eudravigilance Clinical Trial Module (EVCTM), where applicable.

The sponsor will be responsible for the distribution of safety information to Site IRB:

1620 McElderry St., Reed Hall - B130, Baltimore, MD 21205-1911

Faxes:410-955-4367 or 443-287-5353

For questions related to safety reporting, please contact Genentech/Roche Drug Safety:

Tel: (888) 835-2555

Fax: (650) 225-4682 or (650) 225-4630

Other Reports

The sponsor will forward a copy of the Publication to Genentech/Roche upon completion of the Study.

STUDY CLOSE-OUT

Any literature articles that are a result of the study should be sent to Genentech/Roche. Copies of such reports should be mailed to the assigned Clinical Operations contact for the study:

ocrelizumab-iis-d@gene.com , your Genentech MSL and to Genentech Drug Safety CTV oversight mail box at: ctvist_drugsafety@gene.com

QUERIES

Queries related to the Study will be answered by the sponsor. However, responses to all safety queries from regulatory authorities or for publications will be discussed and coordinated between the Parties. The Parties agree that Genentech/Roche shall have the final say and control over safety queries relating to the Product. The sponsor agrees that it shall not answer such queries from regulatory authorities and other sources relating to the Product independently but shall redirect such queries to Genentech/Roche.

Both Parties will use all reasonable effort to ensure that deadlines for responses to urgent requests for information or review of data are met. The Parties will clearly indicate on the request the reason for urgency and the date by which a response is required.

SAFETY CRISIS MANAGEMENT

In case of a safety crisis, e.g., where safety issues have a potential impact on the indication(s), on the conduct of the Study, may lead to labeling changes or regulatory actions that limit or restrict the way in which the Product is used, or where there is media involvement, the Party where the crisis originates will contact the other Party as soon as possible.

The Parties agree that Genentech/Roche shall have the final say and control over safety crisis management issues relating to the Product. The sponsor agrees that it shall not answer such queries from media and other sources relating to the Product but shall redirect such queries to Genentech/Roche.

DISCLOSURE AND PUBLICATION OF DATA

Subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

Upon the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

Data generated by this study must be available for inspection upon request by representatives of national and local health authorities, Genentech, and the IRB for each study site, if appropriate.

The results of this study may be published or presented at scientific meetings. The Sponsor will comply with the requirements for the publication of study results.

Additionally, Section 801 of the Food and Drug Administration Amendments Act (FDAAA 801) (PDF) requires Responsible Parties to register and submit summary results of clinical trials with ClinicalTrials.gov. The law applies to certain clinical trials of drugs (including biological products) and medical devices. (refer to FDAAA 801 Requirements to learn about Responsible Party, Applicable Clinical Trials, and deadlines for registration and results submission)

RETENTION OF RECORDS

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test

results, and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the investigation is discontinued and the U.S. FDA and the applicable national and local health authorities are notified. Genentech will notify the Principal Investigator of these events.

For studies conducted outside the United States under a U.S. IND, the Principal Investigator must comply with U.S. FDA IND regulations and with the record retention policies of the relevant national and local health authorities.