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STATISTICAL ANALYSIS PLAN

TITLE: **A PHASE IV MULTICENTER, OPEN-LABEL STUDY EVALUATING B CELL LEVELS IN INFANTS OF LACTATING WOMEN WITH CIS OR MS RECEIVING OCRELIZUMAB – THE SOPRANINO STUDY**

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STATISTICAL ANALYSIS PLAN APPROVAL

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ADID	Average Oral Daily Infant Dosage
AE	Adverse Event
AESI	Adverse Events of Special Interest
AIRI	Antibody Immune Response Analysis Set of Infants
ALT	Alanine Transaminase
ASQ	Ages and Stages Questionnaire
AST	Aspartate Transaminase
ATC	Atomic Therapeutic Chemical
AUC	Area Under the Milk Concentration-Time Curve
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
CDISC	Clinical Data Interchange Standards Consortium
CI	Confidence Interval
CIS	Clinically Isolated Syndrome
CNS	Central Nervous System
CTCAE	Common Terminology Criteria for Adverse Events
DMT	Disease Modifying Therapy
DTaP	Diphtheria, Tetanus, and Pertussis
eCRF	Electronic Case Report Form
EDSS	Expanded Disability Status Scale
FAS	Full Analysis Set
FASI	Full Analysis Set Infants
FASM	Full Analysis Set Mothers
HBcAb	Hepatitis B Core Antibody
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
Hib	Hemophilus Influenzae type b
ICE	Intercurrent Event
Ig	Immunoglobulin
IRR	Infusion-Related Reaction
IU	International Unit
LLN	Lower Limit of Normal
LMP	Last Menstrual Period
mAb	Monoclonal Antibody
MDID	Maximum oral Daily Infant Dosage
MedDRA	Medical Dictionary for Regulatory Activities
MMR	Measles, Mumps, and Rubella
MS	Multiple Sclerosis
NCI	National Cancer Institute

Abbreviation	Definition
NK	Natural Killer
PASI	Pharmacokinetic Analysis Set Infants
PASM	Pharmacokinetic Analysis Set Mothers
PCV-13	13-Valent Pneumococcal Conjugate Vaccine
PK	Pharmacokinetic
PPMS	Primary Progressive Multiple Sclerosis
PT	Preferred Term
RBC	Red Blood Cell
RRMS	Relapsing Remitting Multiple Sclerosis
SAE	Serious Adverse Event
SAFI	Safety Analysis Set of Infants
SAFM	Safety Analysis Set of Mothers
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
SPMS	Secondary Progressive Multiple Sclerosis
TEAE	Treatment-Emergent Adverse Event
US	United States
WBC	White Blood Cell
WHO	World Health Organization

1. BACKGROUND

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating and degenerative disease of the central nervous system (CNS) that affects approximately 1 million people in the United States (US) and 2.8 million worldwide.

Ocrelizumab is a recombinant humanized monoclonal antibody (mAb) that selectively targets and eliminates CD20-expressing B cells, which are believed to play a critical role in MS. Efficacy and safety of ocrelizumab has been demonstrated in one Phase II and three Phase III randomized controlled clinical trials.

Given the unmet need for women with MS who may wish to breastfeed but are at high risk of post-partum relapses, and given the available evidence of minimal transfer of rituximab into breastmilk, a dedicated prospective interventional study to specifically evaluate the transfer of ocrelizumab into breastmilk and the corresponding pharmacodynamic effects in infants is therefore required, and justified. This study (SOPRANINO) is part of the Sponsor's broader research effort to investigate the benefit-risk of exposure to ocrelizumab during pregnancy and lactation.

2. STUDY DESIGN

This is a prospective, multicenter, open-label study in lactating women with clinically isolated syndrome (CIS) or MS (in line with the locally approved indications) who decided together with their treating physician to continue on, or start treatment with, OCREVUS® (ocrelizumab) post-partum despite ocrelizumab currently not being recommended during lactation.

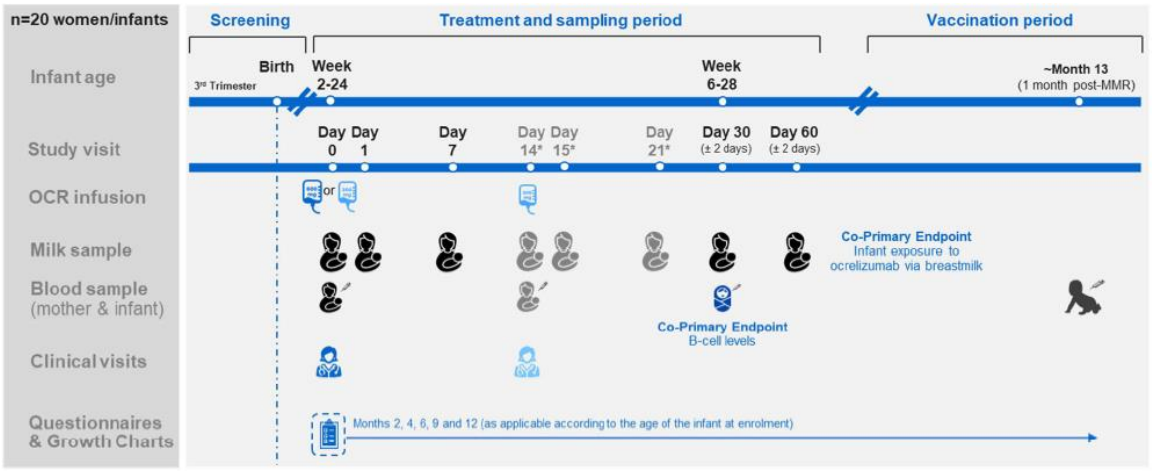
This study will enroll at least 10 women with CIS or MS who are breastfeeding or planning to breastfeed. Laboratory and clinical assessments will be performed as described in the Schedule of Assessments presented in Appendix 2.

The study will consist of the following periods:

- Screening period
- Treatment and sampling period
- Vaccination period

Figure 1 presents an overview of the study design. The schedule of assessments is provided in Appendix 2.

Figure 1 Overview of Study Design



Samples and visits at Days 14, 15 and 21 apply only to patients initiating treatment with ocrelizumab at 2 × 300 mg separated by a 14-day interval.

ASQ-3 = Ages and Stages Questionnaire, version 3; CIS = Clinically isolated syndrome; LMP = last menstrual period; MS = Multiple Sclerosis; OCR = Ocrelizumab.

Study description: In this prospective, multicenter, open-label study, lactating women with CIS or MS (in line with the locally approved indications) who decided together with their treating physician to continue on, or start treatment with, commercial ocrelizumab post-partum will enter a **screening period**, which may be started during the third trimester of pregnancy and continue until 24 weeks post-partum. Women who have delivered a *healthy* term infant and made a decision to breastfeed their infant will be enrolled if they and their infants fulfil the respective eligibility criteria. Women resuming treatment with ocrelizumab post-partum will be included only if the last exposure to ocrelizumab occurred more than 3 months before the LMP (i.e., women without potential fetal exposure). In the **60-day (±2 days) treatment and sampling period**, women will receive the ocrelizumab dose regimen as per the locally-approved label, *at any point* between Week 2 and Week 24 post-partum. The first dose of ocrelizumab may be administered as two 300-mg infusions separated by 14 days (for those initiating ocrelizumab) or as a single 600-mg infusion (for those resuming ocrelizumab). For women where a decision not to administer a second 300 mg infusion is taken after enrolment, continuation in the study will be allowed. Women referred by healthcare professionals to participate in the trial may receive ocrelizumab treatment at their neurologist's site as part of their standard of care treatment. Laboratory and clinical assessments will be performed at the designated visits.

Women will collect their breastmilk samples over several time points up to 60 (±2) days after the first post-partum ocrelizumab infusion, reserving 5 mL at each sampling point for analysis of ocrelizumab concentrations. Infant blood samples will be collected at Day 30 (±2 days) after the first post-partum ocrelizumab infusion (regardless of whether women receive a 600 mg or a 2 × 300 mg dose). Infant blood samples may be collected at home by a visiting nurse, if not collected at the clinical site during a study visit. In **the vaccination period,** infants will continue to be followed-up for growth and developmental milestones up to 12 months of age, using appropriate growth charts, absolute values and the ASQ-3 questionnaire (other standard measurements recorded by for example, the pediatrician as part of routine postnatal care, may also be used). Infant laboratory assessments will be performed 1 month (+ 30 days) after the first dose

of MMR vaccine (if first dose is administered at 11 months of age or later) or 1 month (+ 30 days) after second dose of MMR vaccine (if first dose is administered before 11 months of age), or at Month 13 of chronological age (+ 30 days) if MMR vaccine is not planned to be administered, to evaluate whether infants are able to mount humoral immune responses to clinically-relevant vaccines, and for measurement of B-cell levels. A structured telephone interview will be conducted by site personnel post-partum every 3 months (in-between ocrelizumab infusions) for a general review, and to identify and collect information on any changes in the woman's or infant's health status (including the occurrence of MS relapses in the mother and use of new concomitant medications) and possible adverse events in both the woman and the infant (particularly infections); women will also be asked if the ASQ-3 form is being filled out. No telephone contact is needed in weeks where the woman is performing on-site visits. Women who decide to discontinue the study (this includes discontinuation of either the mother or the infant) will be invited to attend an early study discontinuation visit as soon as possible (this visit may be conducted virtually or by telephone).

Further details of the procedures during each period can be found in the protocol.

2.1 PROTOCOL SYNOPSIS

The Protocol Synopsis is in Appendix 1 – please refer here for the list of objectives and corresponding endpoints. For additional details, see the Schedule of Assessments in Appendix 2.

2.2 DETERMINATION OF SAMPLE SIZE

There is no formal sample size calculation, as no confirmatory hypothesis testing is planned. The primary analysis will be descriptive. The study will include at least 10 women with CIS or MS (in line with the locally approved indications) who are breastfeeding or planning to breastfeed.

With 10 infants, a precision (width of the two-sided 95% CI) of 0.443 is expected if one event is observed (defined as B cells below the lower limit of normal [LLN]) and a precision of 0.531 if two events are observed in the study. If no event is observed during the study, there is a 95% confidence that the event rate is below 0.31.

2.3 ANALYSIS TIMING

The primary analysis will be conducted after the last breastmilk sample collection at the end of the 60-day Treatment and Sampling Period.

The full analysis, including analysis of the vaccination response in infants, will be conducted at the end of the study. The end of the study is defined as the date of the last assessment (vaccine response titers measured 1 month (+30 days) after the first or second dose of Measles Mumps and Rubella (MMR) vaccine, or at month 13 of age [+30 days] if MMR vaccine is not planned to be administered) for the last infant.

No formal effectiveness and safety interim analyses are planned. Interim analyses for administrative or scientific purposes may be conducted during the course of the study.

3. STUDY CONDUCT

3.1 RANDOMIZATION

No randomization, treatment assignment or blinding is planned.

3.2 INDEPENDENT REVIEW FACILITY

Not applicable to this study.

3.3 DATA MONITORING

There is no Independent Data Monitoring Committee (IDMC) planned for this study.

4. STATISTICAL METHODS

The analysis of this open-label study will be primarily based on descriptive statistical methods. Unless otherwise specified, statistical tests will be exploratory in nature. Corresponding 95% confidence interval (CI) will be presented as appropriate. No correction for multiple testing will be applied.

For continuous variables, descriptive statistics (e.g., number of patients [n], mean, standard deviation [SD], median, 25th and 75th percentiles, minimum, maximum) will be calculated and summarized.

For categorical variables, the number and percentage in each category will be displayed.

Unless specified otherwise, values at the mother's first ocrelizumab infusion visit (which may take place any time between Week 2 and Week 24 post-partum) will be considered as baseline for all analyses. For analyses in the infant, date of birth will be considered as baseline.

Note that the protocol's Schedule of Assessments (see Appendix 2) defines the day of first post-partum ocrelizumab administration as Day 0, whereas per the Clinical Data Interchange Standards Consortium (CDISC) standards the analysis defines it as Day 1, i.e., Study Day = Date of assessment – Day of first post-partum ocrelizumab dose + 1. Study day will be defined in the footnotes of outputs as appropriate. The primary analysis time point will be described as the "Day 30 Visit" to highlight that it refers to the visit label defined in the protocol rather than the derived study day for analysis, since in this case the derived Study Day = 31.

The analyses outlined in this Statistical Analysis Plan (SAP) supersede those specified in the protocol.

4.1 ANALYSIS SETS

4.1.1 Screened Set (Mothers)

All mothers who had informed consent signed.

4.1.2 Enrolled Set (Mothers)

All mothers who were enrolled into the study.

4.1.3 Enrolled Set (Infants)

All infants who were enrolled into the study.

4.1.4 Full Analysis Set (Mothers)

The full analysis set of mothers (Full Analysis Set Mothers [FASM]) will include all enrolled mothers who receive any post-partum dose of ocrelizumab.

4.1.5 Full Analysis Set (Infants)

The full analysis set of infants (FASI) will include all the infants of women in the FASM population.

4.1.6 Full Analysis Set (Mothers & Infants)

The full analysis set (FAS) will include the combined population of infants and mothers in the FASI and FASM respectively.

4.1.7 Pharmacokinetic-Analysis Set (Mothers)

The pharmacokinetic (PK) analysis set of mothers (PASM) will include all women in the FASM with a sufficient number of ocrelizumab concentration samples from breastmilk to allow calculation of PK parameters. This analysis set will be used to estimate the PK parameters of ocrelizumab in the breastmilk of lactating women with CIS or MS receiving ocrelizumab post-partum.

4.1.8 Pharmacokinetic-Analysis Set (Infants)

The PK analysis set of infants (PASI) will include all infants in the FASI with a serum sample to allow measurement of ocrelizumab concentration. This analysis set will be used to estimate the serum concentration of ocrelizumab in the infant due to ocrelizumab transfer from the mother to the infant via breastmilk.

4.1.9 Safety Analysis Set (Mothers)

The safety analysis set of mothers (SAFM) will be the same as the FASM.

4.1.10 Safety Analysis Set (Infants)

The safety analysis set of infants (SAFI) will be the same as the FASI.

4.1.11 Antibody Immune Response Analysis Set (Infants)

The antibody immune response analysis set of infants (AIRI) will include all infants in the SAFI for whom any serum titers of antibody immune response to vaccinations are available. This analysis set will be used to analyze humoral immune responses to common childhood immunizations.

4.2 ANALYSIS OF STUDY CONDUCT

Patient disposition information will be summarized separately for the mother and infant, as: the reason and number of screen failures (mother only), the number enrolled, in the respective FAS population, in the respective safety analysis set (SAF) population, completed the treatment and sampling period, discontinued from treatment and sampling period and the reasons for discontinuation, completed the vaccination period, discontinued study and the reasons for study discontinuation.

Disposition information will be listed for mother/infant pairs in a single combined listing.

Mothers and infants who discontinued from the treatment and sampling period will be listed including reasons for discontinuation.

Time on study (weeks) will be summarized for mothers and infants, and calculated as: (date of last assessment* – date of mother's 1st post-partum ocrelizumab dose + 1) / 7.

* Date of last assessment for a subject is defined as the maximum of study completion/discontinuation date and the latest recorded date in all CRF and non-CRF data.

Major protocol deviations, including deviations of inclusion/exclusion criteria, will be summarized in the FAS of mother/infant pairs.

The number enrolled by site and country will be summarized in the enrolled population of mother/infant pairs.

4.3 Demographic and Baseline Characteristics

4.3.1 Mothers

The following demographic, MS history, baseline disease characteristics of mothers will be summarized and listed in the FASM, unless otherwise stated:

Demographic and Baseline Characteristics

- Age at screening (years) as continuous and categorical (18 to ≤30, >30 to ≤35, >35 to ≤40, >40)
- Self-reported race and ethnicity
- Height (cm), weight (kg), and Body Mass Index (BMI: kg/m²)
- Smoking history (never, current, former)
- If previous smoker, time (years) since last use

- Alcohol use history (never, current, former)
- If previous user of alcohol, time (years) since last use
- Number of weeks post-partum
- Fertility status (listing only)

Multiple Sclerosis History

- Medical condition (Relapsing Remitting Multiple Sclerosis [RRMS], Secondary Progressive Multiple Sclerosis [SPMS], Primary Progressive Multiple Sclerosis [PPMS], CIS)
- Duration (years) since onset of MS symptoms and its category ($\leq$3 years, >3 to $\leq$5 years, >5 years to $\leq$10 years, >10 years to $\leq$15 years, > 15 years)
- Duration (years) since MS diagnosis and its category ($\leq$ 3 years, >3 to $\leq$5 years, >5 years to $\leq$10 years, >10 years to $\leq$15 years, > 15 years)
- Expanded Disability Status Scale (EDSS) details up to 1 year prior to pregnancy
- Number of EDSS assessments and its category (0, 1, $\geq$2)
- Most recent EDSS score
- Change in EDSS score from last pre-baseline measurement (up to 1 year before LMP) to baseline

Multiple Sclerosis History - Relapse

- Number of relapses up to 1 year prior to pregnancy and its category (0, 1, 2, 3 and $\geq$ 4)
- Women with relapses up to 1 year prior to pregnancy (Yes, No)
- Duration (months) since last onset of MS relapse prior to enrollment and its category ($\leq$6 months, $>$6 and $\leq$12 months, $>$12 and $\leq$18 months, $>$18 months)

If the date of the relapse is completely missing or if only the year is available, the patient will be excluded from the analysis as appropriate. If the day is missing but the month is available, it will be imputed to the first day of the month.

Multiple Sclerosis History - Prior Disease Modifying Therapies Use

- Number of Disease Modifying Therapies (DMTs) used prior to pregnancy (1, 2, 3 and >3)
- Type of DMTs used prior to pregnancy
- Number of DMTs used during pregnancy (1, 2, 3 and $>$3)
- Type of DMTs used during pregnancy
- Number of DMTs used post-partum but before baseline (1, 2, 3 and >3)

- Type of DMTs used post-partum but before baseline
- Number of patients by the most recent DMT
- Reason for discontinuing most recent DMT
- Duration of the most recent DMT as continuous and categorical (≤6 months, >6 and ≤12 months, >12 and ≤24 months, >24 months)
- Time (months) from last DMT to LMP prior to study
- Time (months) from last DMT to first ocrelizumab prior to study

Multiple Sclerosis History - Prior Ocrelizumab Use

- Received ocrelizumab prior to the study (Yes, No)
- Duration (months) of ocrelizumab therapy prior to enrolment
- Duration of ocrelizumab therapy prior to enrolment (≤6 months, >6 and ≤12 months, >12 and ≤24 months, >24 months)
- Time (months) between last ocrelizumab infusion and the last menstrual period (LMP) (3 to ≤6 months, >6- to ≤9 months, >9 to ≤12 months, >12 months)
- Time (months) between delivery and first post-partum ocrelizumab infusion

Baseline Disease Characteristics

- Baseline EDSS continuous
- Baseline EDSS distribution: count and percent of patients corresponding to each observed score of EDSS at baseline
- For those who underwent neurological examination at baseline, any new clinically significant abnormalities since last exam (Yes, No)
- Type of abnormality for those with clinically significant abnormalities since last exam (disability progression independent of relapse, MS relapse, signs or symptoms suggestive of Progressive Multifocal Leukoencephalopathy, Other)

Obstetric and Gynecological History

- History of previous pregnancies (Yes/No)
- Number of previous pregnancies (1, 2, 3, 4, >4)
- Previous pregnancy outcome (Full term live birth, preterm live birth, spontaneous abortion, still birth, therapeutic abortion, elective abortion, ectopic pregnancy, unknown)
- History of obstetrical complications during previous pregnancies (Yes/No)

Medical History

Medical history of the mother will be summarized in the SAFM by SOC and PT. For each previous and concurrent medical history recorded medical history (MH), the term entered

by the investigator describing the event (the “reported term”) will be assigned a standardized term (the “Preferred Term” [PT]) and assigned to a superclass term (the “System Organ Class” [SOC]) on the basis of the MedDRA World Health Organization (WHO) dictionary of terms.

Vaccination History

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination and non-SARS-CoV-2 vaccination history will be summarised in the SAFM overall and split by the period prior to infant’s birth and before/after LMP and after infant’s birth. Where time period cannot be determined due to completely or partially missing date, it will be imputed to before infant’s birth.

SARS-CoV-2 vaccination history will also be listed in the SAFM, including the following:

- Manufacturer name (first, second and, if applicable third dose and any booster dose(s)) (Pfizer/BioNTech Covid-19 vaccine, Moderna Covid-19 vaccine, Astra Zeneca Covid-19 vaccine, Johnson & Johnson Covid-19 vaccine, Other)
- Type of vaccine for each dose (mRNA vaccine, Viral Vector Vaccine, Inactivated Vaccine, Other)
- Timepoint of last COVID-19 vaccination prior to enrollment (>6 months before pregnancy, ≤6 months before pregnancy, 1st trimester, 2nd trimester, 3rd trimester, ≤6 months post-partum, >6 months post-partum)

Other vaccination history (vaccines other than SARS-CoV-2) will also be listed in the SAFM.

4.3.2 Infants

The following baseline characteristics of infants will be summarized and listed in the FASI, unless otherwise stated:

Characteristics at Birth

- Details of birth (vaginal delivery, vaginal delivery - forceps/vacuum instrumental, cesarean – scheduled, cesarean – emergency)
- Gestational age (weeks)
- Sex (male, female)
- Weight (kg)
- Length (cm)
- Head circumference (cm)
- Apgar score – 1 minute
- Apgar score – 5 minute
- Apgar score – 10 minute

- Congenital anomalies

Characteristics at Screening

- Age (weeks)
- Sex (male, female)
- Weight-for-Age percentile categories
- Length/Height-for-Age percentile categories
- Head Circumference-for-Age percentile categories

Characteristics at Baseline

- Sex (male, female)
- Age (weeks)
- Exposure via breastmilk (months)

Medical History

Medical history of the infant will be summarized in the SAFI by System Organ Class (SOC) and preferred term (PT), following the same approach as previously described for medical history of the mother.

Vaccination Detail

Vaccination details of the infant will be listed in the SAFI.

4.4 PRIMARY ENDPOINTS ANALYSIS

4.4.1 Co-Primary Endpoints

The co-primary endpoints are:

1. Proportion of infants with B cell levels (CD19+ cells, absolute counts in blood) below the LLN, measured at Day 30 Visit after the mother's first ocrelizumab post-partum infusion
2. Estimated average oral daily infant dosage (ADID), calculated as the ocrelizumab average milk concentration over 60 days multiplied by an estimated infant milk intake of 150 mL/kg/day.

The estimand of the proportion of infants with B cell levels below the LLN is defined as:

- Population: All infants of women in the FASM population who have B cell levels measured at Day 30 Visit after the mother's first ocrelizumab post-partum infusion (FASI)

- Variable: Proportion of infants with B cell level (CD19+ cells, absolute counts in blood) below the age-adjusted LLN, measured at Day 30 Visit after the mother's first ocrelizumab post-partum infusion.
- Treatment: Commercial ocrelizumab of the mother (potential exposure via breastmilk).
- Handling of Intercurrent Events (ICEs):
    1. Incomplete dosing, including delayed second (300 mg) ocrelizumab infusion: all the B cell data will be included in the analysis.
    2. Infant did not receive any breastmilk before B cell measurement during the entire 30-day period after the mother's first ocrelizumab post-partum infusion: B cell data will be excluded from the analysis.
    3. Mother used other DMT during breastfeeding before B cell measurement on Day 30 Visit post ocrelizumab infusion 1: B cell data will be excluded from the analysis.
    4. Mother used other medication before B cell measurement on Day 30 Visit post ocrelizumab infusion 1 that is not allowed by protocol during breastfeeding: B cell data will be excluded from the analysis.
    5. Infant's blood sample collected before assessment window (i.e. before day 28, which is before *Analysis* Study Day 29): Data will be excluded from the analysis.
    6. Infant's blood sample collected after the assessment window: B cell data might be included or excluded depending on the cause of the delay and schedule of delayed visit:
        i. Delayed due to operational reason and sample is collected more than 8 days after the assessment window (i.e. > Day 40, which is > Analysis Study Day 41): B cell data will be excluded.

        ii. Delay due to illness of infant: sample collected between 32 days and 40 days while infant has an illness /or recovered from an illness (which is between *Analysis* Study Days 33 and 41): B cell data will be excluded on a case-by-case basis if the illness is likely to confound the B cell data.

    7. Infant's blood sample collected while infant has an illness: B cell data will be excluded on a case-by-case basis if the illness is likely to confound the B cell data.

B-cell reference ranges by week of life (absolute and percentage counts) are defined by Borriello et al. 2022 (Appendix 3). B cell data being confounded with illness will be determined by clinical team in a blinded manner before conducting the primary analysis.

- Population-level summary: The number and proportion of infants with B cell levels below LLN will be reported with the two-sided Clopper-Pearson 95% CI; no formal statistical testing will be done.

The estimand of the ADID in breastmilk is defined as:

- Population: All women in the FASM population who provide any breastmilk samples (PASM).

- Variable: Average oral daily infant dosage (ADID), calculated as the arithmetic mean of the mother's daily ocrelizumab milk concentration over 60 days post ocrelizumab infusion 1 multiplied by an estimated infant milk intake of 150 mL/kg/day. For example, arithmetic mean of the mother's daily ocrelizumab breastmilk concentration over 60 days (A) = 20 ng/mL, assumed fixed daily milk intake (B) = 150 ml/kg, observed weight of infant at Day 30 visit (C) = 3kg. Average oral Daily Infant Dosage (ADID) = A x B x C = 20 ng/mL x 150 mL/kg x 3kg = 9,000 ng = 0.009 mg = 9 μg.

- Treatment: Commercial ocrelizumab.

- Handling of Intercurrent Events (ICEs):
  1. Incomplete dosing, including delayed second (300 mg) ocrelizumab infusion: all the ADID data will be collected and included in the analysis. No adjustments based on actual dose will be made.
  2. Mother used other DMT during breastfeeding: ADID data will be included.
  3. Mother used other medication not allowed by protocol during breastfeeding: ADID data will be included in the analysis.
  4. Mother has mastitis infection during the sample collection period: ADID data will be included from milk samples obtained from the non-infected breast (in case of bilateral mastitis, breastmilk sampling will be interrupted until the infection resolves).

- Population-level summary: Summary statistics together with two-sided 95% CI for the mean will be reported; no formal statistical testing will be done.

4.4.2 Analytical Approach for Co-Primary Endpoints

The number and proportion of infants with B cell levels below the LLN at Day 30 after the mother's first ocrelizumab post-partum infusion will be presented in the FASI, including Clopper Pearson 95% CI. A corresponding listing will be presented for B cell levels (absolute count) at Day 30, including whether any ICEs led to exclusion of subject's data from the analysis.

Summary statistics for the ADID will be presented in the PASM, including 95% CIs for the mean based on the t-distribution. Average oral daily infant dosage will be calculated

over all days for which measurements are available, assuming any missing assessments are missing at random. Breastmilk ocrelizumab concentrations below the limit of quantification (BLQ) will be imputed to zero for the calculation of summary statistics. A corresponding listing will be presented, including whether any ICEs led to exclusion of subject's data from the analysis.

4.4.3 Handling of Missing Data

No data will be imputed unless otherwise stated. Every effort will be made to ensure all samples with all supporting information are collected for B cell measurement.

4.4.4 Sensitivity Analysis for Primary Endpoint

For the primary endpoint of the proportion of infants with B cell levels below the LLN, measured at Day 30 after the mother's first ocrelizumab post-partum infusion, a sensitivity analyses will be performed including those samples collected outside the assessment window as well as those taken while infant has an illness.

4.4.5 Supplementary Analysis for Primary Endpoint

No supplementary analyses are planned for the estimation of proportion of infants with B cell levels below the LLN.

For the estimation of ADID in the breastmilk, the following supplementary analyses may be performed:

- ADID will only be calculated in cases where the complete dose of ocrelizumab was administered. Any subjects experiencing ICE 1 (incomplete dosing) will therefore be excluded from this supplementary analysis.

4.4.6 Subgroup Analyses for Primary Endpoints

Subgroup analyses will be performed for the primary endpoint of the proportion of infants with B cell levels (CD19+ cells, absolute counts in blood) below the LLN, measured at Day 30 after the mother's first ocrelizumab post-partum infusion. Analysis will be presented for the following subgroups:

- The first dose of ocrelizumab post-partum (1x600mg, 2x300mg)
- Type of feed (breast milk, partial breast milk/infant formula)

4.5 SECONDARY ENDPOINTS ANALYSES

4.5.1 B Cell Levels in Infants

B cell levels (CD19+ cells, absolute counts and percentage of lymphocytes) in infants at Day 30 after the mother's first ocrelizumab post-partum infusion will be summarized descriptively in the FASI, irrespective of the occurrence of any ICEs identified for the primary endpoint.

4.5.2 Parameters to Evaluate the Transfer of Ocrelizumab into Breastmilk

The area under the milk concentration-time curve (AUC) of ocrelizumab in mature breastmilk (i.e., milk produced after Day 14 post-partum) over 60 days after the first post-partum ocrelizumab infusion will be calculated using the following time points depending on the dosing schedule:

- If receiving 1x600 mg: before infusion and at 24 hours (Day 1), 7 days, 30 days and 60 days post-infusion
- If receiving 2x300 mg: before infusion 1 and at 24 hours (Day 1), 7 days, 14 days, 15 days (24 hours after infusion 2), 21 days, 30 days and 60 days post-infusion 1

Summary statistics will be presented in the PASM for the following PK parameters of ocrelizumab in the breastmilk measured over 60 days after the mother's first postpartum ocrelizumab infusion, irrespective of the occurrence of any ICEs identified for the primary endpoint:

- Area under the milk concentration-time curve of ocrelizumab in mature breastmilk (AUC)
- Average ocrelizumab milk concentration (C_{mean})
- Peak ocrelizumab milk concentration (C_{max})
- Time to reach peak milk concentration (t_{max}, days)

Summary statistics for each parameter will be presented in the PASM, irrespective of the occurrence of any ICEs identified for the primary endpoint. A corresponding listing will be produced.

For summary stats of all PK parameters other than AUC, concentrations reported as BLQ are set to zero. For AUC calculation, BLQ values before the first measurable concentration are set to zero, and to missing after the last measurable concentration.

Other PK and exposure parameters may be calculated and summarized as appropriate, based on the data obtained.

Subject-level plots of the concentration of ocrelizumab in breastmilk over time will be produced in the PASM.

4.5.3 Relative Infant Dosage (RID) and Maximum Oral Daily Infant Dosage (MDID)

The estimated maximum oral daily infant dosage (MDID) will be calculated at the subject level as the peak ocrelizumab milk concentration measured over 60 days after the mother's first post-partum ocrelizumab infusion multiplied by an estimated infant milk intake of 150 mL/kg/day.

The average relative infant dose (RID, %) over 60 days will be calculated as the ADID (μg) divided by the average maternal dosage (μg) over 60 days multiplied by 100.

i.e.,

Average Oral Daily Infant Dosage (ADID) (A) = 9 μg
Average maternal dosage over 60 days (B) = total dose / 60 = 600 mg / 60 = 10 mg = 10,000 μg
Average RID (%) based on ADID = (A / B) x 100 = (9 / 10,000) x 100 = 0.09%

The maximum RID (%) will also be calculated, where MDID is substituted for ADID in the formula above, i.e.

Maximum RID (%) = (MDID / Average maternal dosage over 60 days) x 100

Breastmilk ocrelizumab concentrations reported as BLQ will be imputed to zero for the calculation of summary statistics.

Summary statistics for MDID, average RID and maximum RID will be presented in the PASM, irrespective of the occurrence of any ICEs identified for the primary endpoint.

The analysis will be repeated for the following subgroups:

- The first dose of ocrelizumab post-partum (1x600mg, 2x300mg)

4.5.4 Infant Serum Concentration of Ocrelizumab

To evaluate whether there is transfer of ocrelizumab from the mother to the infant via breastmilk, serum concentrations of ocrelizumab in the infant are measured 30 days after the mother's first ocrelizumab post-partum infusion.

Ocrelizumab concentrations reported as BLQ will be imputed to zero for the calculation of summary statistics.

Summary statistics for the serum concentration of ocrelizumab in the infant will be presented in the PASI, and a corresponding listing produced.

4.5.5 Infant Absolute Antibody Immune Response

The infant's antibody (immunoglobulin [IgG]) immune response to common childhood immunizations with full or partial doses given prior to 1 year may include (but is not limited to) responses to MMR, diphtheria, tetanus, and pertussis (DTaP), Hemophilus influenzae type b (Hib), hepatitis B virus (HBV) and 13-valent pneumococcal conjugate vaccine (PCV-13), measured 1 month after the first or second dose of MMR vaccine or at Month 13 of age (+30 days) in case MMR vaccine is not planned to be administered.

Summary statistics for each serum IgG antibody titer will be presented in the AIRI, and a corresponding listing produced. Results for MMR titers and other vaccine induced antibodies will be summarized together irrespective of whether or not the infant received MMR vaccine. Listings will include whether or not the mother switched to another DMT regardless of timing.

4.5.6 Infant Positive Antibody Immune Response

The number and proportion of infants with positive humoral response (seroprotective titers as defined for the individual vaccine) will be presented for each IgG antibody titer in the AIRI population. Analysis will be presented by total as well as by women not switching to another DMT vs switching to another DMT regardless of timing.

A corresponding listing will be produced.

Seroprotective titers are defined for each vaccine in the table below:

Vaccine test	Seroprotective Titer
Bordetella pertussis antibodies, IgG	> 1.04 COI
Haemophilus influenzae B, IgG	≥ 0.15 μg/mL
Anti-Diphtheria IgG(-70)CL	≥ 0.01 IU/mL
Anti-Tetanus Toxoid IgG(-70)RUO	≥ 0.01 IU/mL
13 Valent anti-pneumococcal antibody panel	≥ 0.35 μg/mL
Anti-Measles Vir IgG(-70)CL	≥ 120 mIU/mL
Anti-MumpsAT Vir iGG(-70)CL	≥ 17 U/mL
Anti-Rub Vir IgG(-70)RUOCL	≥ 10 IU/mL
Anti-HBs	≥ 10 mIU/mL

COI=Cut-off Index; IU=International Unit IgG=immunoglobulin G.
Source: Storsaeter et al. 1998; EMA 2015.

For Tetanus and Diphtheria, a sensitivity analysis will be conducted using a seroprotective threshold of ≥ 0.1 International Unit (IU)/mL for infants receiving a 4th dose at least one month prior to the titer assessment. The ≥0.01 IU/mL threshold will still be used for those only receiving 3 doses.

4.6 EXPLORATORY ENDPOINTS

4.6.1 Infant Growth Velocity

Infant growth velocity will be assessed by recording the infant's weight (kg), height (cm) and head circumference (cm) at birth, and Months 2, 4, 6, 9, and 12. Analysis will be based on the SAFI and includes:

- Actual values and change from baseline (defined as at birth) for weight, length/height, head circumference, and growth velocity (defined as the change

from baseline divided by the number of months since baseline) will be summarized at each age time-point (birth, and Months 2, 4, 6, 9 and 12).

- The weight-for-age, length/height-for-age, and head circumference-for-age percentiles and change from baseline percentiles at each time-point will be summarized.
- The number and percentage of infants in the 3rd, 5th, 10th, 25th, 50th, and >50th percentile of the WHO growth charts will be presented for weight-for-age, length/height-for-age, and head circumference-for-age percentiles at each age time point. The number and percentage of infants within 10^{th}-90^{th} percentile, and 3rd-97th percentile of the WHO growth charts will also be presented.
- Shift tables will be presented for each parameter to compare the change from infant's percentile at baseline (≤ 3rd, >3rd - ≤5th, >5th - ≤10th, >10th - ≤25th, >25th -≤50th, >50th) to each age time-point (WHO 2006; WHO 2007; WHO 2017).
- Mean percentiles for weight-for age, length/height-for-age, and head circumference-for-age and corresponding 90% CIs will be plotted over time.
- Individual growth charts will be presented for each infant. This chart will present the weight, length/height, and head circumference-for-age for the infant over time, including reference lines for the 3rd, 25th, 50th, 75th, 90th and 97th percentiles for weight-for-age, length/height-for-age, and head circumference-for-age from WHO growth charts. Separate plots will be produced for each infant. Combined plots will also be produced for males and females separately, to show the trajectories of each infant on the same page.

All observed assessments will be included in the analysis; no imputation for missing assessments will be performed. Analyses will also be presented for the following subgroups:

- Type of feed: exclusive breast milk since birth to month 6 vs others (including complementary feeding [breastmilk + formula milk, in various proportions] and formula feeding [no breast milk]).

All infant growth velocity data will be listed.

4.6.2 Childhood Developmental Milestones

The infant's developmental milestones will be assessed using the Ages and Stages Questionnaire version 3 (ASQ-3), in the domains of communication, gross motor, fine motor, problem solving, and personal-social at months 2, 4, 6, 9, and 12.

The total score for each domain will be summarized by visit using summary statistics in the SAFI.

In addition, the number and percentage of infants with abnormal scores (Yes/No) will be presented in the SAFI, as per cut-offs below transcribed from the ASQ-3 User's guide:

ASQ-3 questionnaire	Area				
	Communication	Gross Motor	Fine Motor	Problem Solving	Personal-Social
Month 2	22.77	41.84	30.16	24.62	33.71
Month 4	34.60	38.41	29.62	34.98	33.16
Month 6	29.65	22.25	25.14	27.72	25.34
Month 9	13.97	17.82	31.32	28.72	18.91
Month 12	15.64	21.49	34.50	27.32	21.73

ASQ-3 = Ages and Stages Questionnaire version 3.

Values below the respective cut-off will be classified as abnormal (i.e., further assessment with a professional may be needed).

A corresponding listing will be produced.

Only questionnaires assigned to the correct time point will be included in the main analysis. If the wrong questionnaire is used for some time points in >20% infants, a sensitivity analysis will be performed with these data included.

4.6.3 Women's Disease Activity

To assess disease activity in women the EDSS score, a neurological examination and potential relapses are collected at baseline, early discontinuation and unscheduled visits if applicable. This will be summarized as follows in the FASM:

- Relapse:
  - Number of relapses before/during pregnancy (defined by LMP) and after pregnancy (defined as after birth) will be summarized in 3-month intervals
  - Number and percentage of women in each category (0, 1, 2, 3 and $\geq$4) in 3-month intervals of before/during/after pregnancy
  - Among women who received their last ocrelizumab within 12 months before the LMP:
    - Number of relapses between 6-9 months after last ocrelizumab dose before Baseline
    - Number of relapses in the 3 months after the first post-partum ocrelizumab dose.
  - Annualized relapse rate and 95% CI may also be presented in 3-month intervals if warranted by the number of data points

- Presence of any clinically significant abnormalities from the neurological examination (Yes, No)
- Type of neurological abnormality (disability progression independent of relapse, MS relapse, signs or symptoms suggestive of Progressive Multifocal Leukoencephalopathy, Other)

Corresponding listings will be produced for relapses, EDSS, neurological examination findings.

4.6.4 Infant Evolution of B Cell Levels

To evaluate the evolution of B cell levels over the first year of life, B cell levels (CD19+ cell, absolute counts and percentage of lymphocytes) are collected in the infant at Day 30 Visit and 1 month after the first dose of MMR vaccine or at Month 13 of age if MMR vaccine is not planned to be administered.

Summary statistics for the mean absolute values and percentage change from Day 30 will be presented at each visit (Day 30, 1 month after first MMR vaccine, Month 13 of age if MMR vaccine is not administered) for absolute CD19+ cell counts and percentage of lymphocytes based on the FASI, irrespective of the occurrence of any ICEs identified for the primary endpoint.

The proportion of infants with CD19+ B cell levels below LLN, and summary statistics for the result, will be presented by visit (Day 30, Month 13).

Subject profile plots (one line per infant) will be used to display the trajectory of CD19+ cell levels (absolute counts and percentage of lymphocytes) over time in the FASI. The Y axis will be the absolute cell count or percentage of lymphocytes, and the X axis will be age in weeks. Lines will be plotted based on the actual age (i.e., not pooled into age categories). The age-specific LLN and ULN (see Appendix 3) for CD19+ cell counts and percentage of lymphocytes will be overlayed.

All B cell data collected will be listed. This listing will flag cases where absolute cell counts at each visit fall below the age-specific LLN for CD19+ (based on Borriello et al. 2022 [see Appendix 3]), and for CD3, CD4, CD9 and CD16+56 (based on Tosato et al. 2015 [see Appendix 4]).

4.6.5 Feeding Schedule / Feeding Status

The following Feeding Schedule Diary data will be presented by Study Day in the FASM (noting that Study Days 13, 14, 15, 16, 20, 21 and 22 should only be completed for subjects who receive 2×300 mg ocrelizumab infusions in 2 separate visits):

- Number and proportion with each type of feed (breastmilk, partial breastmilk/infant formula, other feeds such as baby food)
- Mean number of breastmilk feeds
- Mean number of formula milk feeds

- Mean number of other feeds (baby food)

After the 60-day sample period, woman may switch to another DMT and continue to breastfeed, this will be summarized as follows in the FASM:

- Number and proportion with each type of feed (breastmilk, partial breastmilk/infant formula, other feeds such as baby food) at Months 2, 4, 6, 9, and 12, by whether or not they switched to another DMT, regardless of timing

A corresponding listing will be produced.

4.7 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

Pharmacokinetic and pharmacodynamic (B cells) analyses for ocrelizumab in the breastmilk and serum are described elsewhere for the primary and secondary endpoints (see Sections 4.4 and 4.5).

4.8 SAFETY ANALYSES

The safety outcome measures to be analyzed comprise the following: incidence and nature of all adverse events (AEs), including findings on vital sign measurements, neurological examinations, clinical laboratory tests, and concomitant medications.

All safety analyses will be conducted on applicable data collected from the first post-partum ocrelizumab infusion until the end of the study. The safety analysis will be performed on the SAFM and infants (SAFI) separately.

4.8.1 Exposure of Study Medication

Definitions

Dose: Ocrelizumab dose is given as one infusion or two infusions administered 2 weeks apart. Patients will be considered to have received a Dose of treatment if at least part of one infusion of that Dose (either Day 1 or Day 15 for dual infusions) was given.

Treatment duration (in weeks) for a woman will be calculated as follows:

[(Date of last contact* − Date of first post-partum dose) + 1] / 7

*Earliest 1) date of last OCR dose plus risk window of 6 months (182 days)
2) date of death
3) clinical cut-off date

Exposure Analysis

The following measures of exposure of ocrelizumab will be summarized in the SAFM using descriptive statistics:

- Number of patients completing each dose type (Dose 1, full dose; Dose 1 split dose #1; Dose 1, split dose #2)
- Treatment duration (weeks)

- Number of doses received (Day 1 and Day 15 infusions are considered as one dose)
- Total cumulative dose (mg)

A corresponding listing will be produced.

The analysis of exposure to ocrelizumab in infants (as measured via concentration in the breastmilk, and also directly from serum samples) is described previously as part of the primary and secondary endpoints.

4.8.2 Adverse Events

All summaries of AEs will be based on the respective safety population for mothers and infants, and will include AEs occurring between the mother's first post-partum ocrelizumab infusion and the end of the study. Only treatment-emergent AEs (TEAEs) will be analyzed and the terms TEAE and AE will be used interchangeably.

For each recorded adverse event (AE), the term entered by the investigator describing the event (the "reported term") will be assigned a standardized term (the "Preferred Term" [PT]) and assigned to a superclass term (the "System Organ Class" [SOC]) on the basis of the Medical Dictionary for Regulatory Activities (MedDRA) WHO dictionary of terms version 27.1.

Definitions

Treatment-emergent adverse event (TEAE): AEs with an observed or imputed date of onset on or after the start date of trial treatment. If the onset date of the AE is prior to the day of first dose, the AE will be considered treatment-emergent only if the most extreme intensity is greater than the initial intensity (i.e., the intensity for a given AE increases and its end date is on or after the date of the first dose). An AE with a completely missing, non-imputed start date will be assumed to be treatment emergent unless the AE has a complete, non-imputed end date that is prior to the date of the first dose.

Imputation of incomplete date: in order to evaluate if an AE is treatment-emergent, incomplete dates will be imputed as follows:

- If the start date is incomplete:
  - If the day is missing, impute to 01, or to the day of first dose of ocrelizumab if the month and year is the same as the dosing date.
  - If the month is missing, impute to January, or to the month of first dose of ocrelizumab if the year is the same as the year of dosing.
  - If the date is completely missing, then the date will remain missing
- If the end date is incomplete:
  - If the day is missing, impute to the last day of the month
  - If the month is missing, impute to December
  - If the date is completely missing, then the date will be missing

If the imputed start date of an AE is post first dose, it is considered treatment emergent. If the imputed start date of an AE is prior first dose, it is considered non-treatment emergent.

In case of a missing start date and an (imputed) end date prior to first dose, the AE is considered non-treatment emergent.

Otherwise, the AE is considered treatment emergent.

Serious Adverse Events (SAEs): all SAEs including serious MS relapses and serious Infusion Related Reactions (IRRs).

Output Conventions

All analyses of AE data will be performed using the PTs unless otherwise specified.

For all summary tables, the AEs will be sorted by SOC (in decreasing order of overall incidence) and then by PT (in decreasing order of overall incidence). At each level of summarization (at least one event, SOC and PT), patients reporting more than one AE will be counted only once. For TEAEs by grade (intensity), the highest grade will be reported. All AE summaries will be based on TEAEs only.

The following summary tables will be generated for mothers and infants separately:

- Overview summary table of TEAEs, including: total number of subjects with at least one AE, total number of AEs, total number of SAEs, total number of deaths, total number withdrawn from study due to an AE, and total number of subjects with at least one:
    - AE with fatal outcome
    - SAE
    - SAE leading to withdrawal from treatment (“Drug Withdrawn” for the “Action taken with ocrelizumab due to SAE/AE” field in the “Adverse Event/IRR” electronic Case Report Form [eCRF] form) (mothers only)
    - SAE leading to dose modification/interruption (mothers only)
    - Related SAE
    - AE leading to withdrawal from treatment (mothers only)
    - AE leading to dose modification/interruption (mothers only)
    - Related AE
    - Related AE leading to withdrawal from treatment (mothers only)
    - Related AE leading to dose modification/interruption (mothers only)
    - AE of Grade ≥ 3
    - Infusion related reaction (IRR) (mothers only)
    - Serious IRR (mothers only)

- Infections (using the MedDRA SOC of 'Infections and Infestations')
- Serious infections
- Serious infections leading to withdrawal from treatment (mothers only)

- TEAEs, by SOC and PT
- Serious TEAEs, by SOC and PT
- TEAEs, by Highest NCI CTCAE Grade
- Infections, by Type and PT (identified using the Orelizumab specific Adverse Events Glossary of Terms)
- IRRs, by Highest National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) grade (mothers only)
- Infusion Related Reactions by Time of Event (During Infusion, within 24 hours after end of infusion) Overall and by post-partum Infusion (Dose 1 full dose, Dose 1 split dose 1, Dose 1 split dose 2, Dose 2) (mothers only)
- Adverse Events of Special Interest (AESI, mothers only):
  - Cases of potential medicine-induced liver injury that include an elevated alanine transaminase (ALT) or aspartate transaminase (AST) in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's law and in the protocol.
  - Suspected transmission of an infectious agent by the study medicine, as defined in the protocol.

All SAEs, AESIs, AEs leading to withdrawal, infections, and deaths occurring during the study will be listed. For all of these listings, mothers/infants pairs will be presented 'next in line' as one row after the other in the same listing, rather than as two separate listings.

4.8.3 Laboratory Data

Laboratory assessments (performed in central laboratory, except for urinalysis) will include the following:

- **Hematology (Mother)**: hemoglobin, hematocrit, quantitative platelet count, red blood cell [RBC] count, white blood cell [WBC] absolute or/and differential count [neutrophils, eosinophils, lymphocytes, monocytes, basophils].
- **Serum chemistry (Mother):** potassium, sodium, chloride, random glucose, AST, ALT, gamma-glutamyl transpeptidase [GGT], total bilirubin and creatinine.
- **Urinalysis (Mother):** using urine dipstick at site; may include pH, specific gravity, glucose, protein, ketones, blood, and microscopic examination (sediment, RBCs, WBCs, casts, crystals, epithelial cells, bacteria) at the discretion of the investigator.
- **Hepatitis B virus serology (Mother):** Women with positive screening tests for HBV, determined by a positive hepatitis B surface antigen (HBsAg) result (current

infection) or positive hepatitis B core antibody (HBcAb) titers (previous infection) will be excluded. Women with documented history of HBV vaccination or positive HBsAb titers are eligible.

- **Serum immunoglobulin (Ig) concentration (Mother):** Quantitative measurement for IgG, IgM and IgA levels.
- **Serum titers (IgG) of antibody immune responses to vaccinations (Infant):** May include but not be limited to MMR, DTaP, Hib, HBV and PCV-13; 1 month after the first or second dose of MMR vaccine, or at Month 13 of age (+30 days) if MMR vaccine is not planned to be administered.
- **Lymphocyte subtypes (Mother & Infant):** Blood samples will be collected to measure B-cell counts (CD19+ and B-cell subsets** [see below]), T-cell counts (CD3+, CD4+, CD8+), and natural killer (NK) cell counts (CD16+CD56+).

  **B-cell subsets:
  Naïve B cells: CD45+, CD19+, IgD+, CD27-, CD38dim/-
  Memory B cells: CD45+, CD19+, CD27+
  Unswitched Memory B cells: CD45+, CD19+, IgD+, CD27+
  Switched memory B cells: CD45+, CD19+, IgD-, CD27+
  Double Negative B cells: CD45+, CD19+, IgD-, CD27-
  IgD Transitional B cells: CD45+, CD19+, IgD+, CD27-, $CD38^{bright}$
  Plasmablasts/Plasma cells: CD45+, CD19+, CD27+, $CD38^{bright}$

4.8.3.1 General Laboratory Evaluation and Reporting Conventions

All laboratory assessments in the mother (hematology, serum chemistry, urinalysis, Hepatitis B virus serology, serum immunoglobulin concentration) will be summarized in the SAFM or listed when applicable.

Absolute value and change from baseline at each visit will be summarized using standard unit. The baseline value for laboratory assessments summarized in mothers will be the last value prior to the first dose of study medication. When laboratory data are presented over time, these laboratory values will be time-windowed into a common visit structure. If multiple values of the same laboratory parameter occur within the same time window, the worst value for that parameter will be presented in the summary table.

Unless specified otherwise, any laboratory parameters which are below the BLQ will be listed, for summaries a value of the LLQ/2 will be imputed. Values above the upper limit of quantification will be summarized at the upper limit of quantification.

For hematology and serum chemistry, the number and percentage of patients with normal/abnormal laboratory outcomes based on normal laboratory ranges will be summarized for each parameter and visit. For urinalysis, the number and percentage with each test result category will be reported for each parameter and visit.

Corresponding patient-level listings will be produced for the following in the SAFM: hematology, serum immunoglobin concentration, laboratory abnormalities for hematology, laboratory abnormalities for serum chemistry, laboratory abnormalities for urinalysis.

4.8.3.2 Lymphocytes Evaluation

All lymphocytes parameters in the infants (with the exception of CD19+ cells already covered in the primary and secondary endpoint analyses) will be summarized using absolute count and change from baseline values at each visit. All lymphocytes parameters in the mothers at screening will be summarized.

Corresponding listings will also be produced for lymphocyte parameters in infants and mothers.

Parameters may also be displayed graphically over time, as appropriate.

4.8.3.3 Serum Antibody Titers Evaluation

The secondary endpoint analysis of serum titers (IgG) of antibody immune responses to vaccinations in the infant is described in Section 4.5.5.

4.8.4 Vital Signs

The actual value and change from Baseline at each visit will be summarized for the following vital signs parameters in mothers: systolic blood pressure (mmHg), diastolic blood pressure (mmHg), pulse rate (beats/min), temperature (ºC).

4.8.5 Prior and Concomitant Medications

Prior (defined as treatment ended before mother's first dose of study drug) and Concomitant medications (defined as treatment used at any time since mother's first dose of study drug) will be summarized, for mothers and infants separately, by frequency tables according to the Anatomic Therapeutic Chemical (ATC) classification system using the WHO Drug dictionary.

Pre-infusion prophylactic treatment will be summarized by medication class in mothers, by frequency tables according to the ATC classification system using the WHO Drug dictionary.

Corresponding listings will also be produced.

4.9 MISSING DATA

All methods for handling missing data and associated sensitivity analyses are described above, section by section, for each endpoint. If not otherwise specified, missing values are not imputed.

4.10 INTERIM ANALYSES

No formal effectiveness and safety interim analyses are planned. Interim analyses for administrative or scientific purposes may be conducted during the course of the study.

5. CHANGES TO PLANNED ANALYSIS

The following list documents changes between the protocol-defined statistical analyses and those presented in the SAP:

1. The protocol refers to the PASM as the Primary Analysis Set Mother. This definition was corrected in the SAP to Pharmacokinetic Analysis Set Mother.

6. REFERENCES

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Appendix 1 Protocol Synopsis

TITLE:	**A PHASE IV, MULTICENTER, OPEN-LABEL STUDY EVALUATING B-CELL LEVELS IN INFANTS OF LACTATING WOMEN WITH CIS OR MS RECEIVING OCRELIZUMAB – *THE SOPRANINO STUDY***
PROTOCOL NUMBER:	MN42989
VERSION NUMBER:	3
EUDRACT NUMBER:	2021-000063-79
IND NUMBER:	100593
NCT NUMBER:	NCT04998851
TEST PRODUCT:	Ocrelizumab (RO4964913)
PHASE:	Phase IV
INDICATION:	Multiple Sclerosis
SPONSOR:	F. Hoffmann-La Roche Ltd

OBJECTIVES AND ENDPOINTS

This study will evaluate the pharmacokinetics of ocrelizumab in the breastmilk of lactating women with clinically isolated syndrome (CIS) or multiple sclerosis (MS) [in line with the locally approved indications] treated with ocrelizumab, by assessing the concentration of ocrelizumab in mature breastmilk, as well as the corresponding exposure and pharmacodynamic effects (blood B-cell levels) in the infants. Specific objectives and corresponding endpoints for the study are outlined below.

Objectives	Corresponding Endpoints
Co-Primary Outcome Measure	
• To evaluate whether infants of lactating women with CIS or MS receiving ocrelizumab postpartum present with B-cell depletion	• Proportion of infants with B-cell levels (CD19+ cells, absolute counts in blood) below the LLN, measured at Day 30 after the mother's first ocrelizumab postpartum infusion
• To evaluate the exposure to ocrelizumab in infants of lactating women with CIS or MS receiving ocrelizumab postpartum	• Estimated ADID, calculated as the ocrelizumab average milk concentration over 60 days post-ocrelizumab infusion 1 multiplied by an estimated infant milk intake of 150 mL/kg/day
Secondary Outcome Measures	
• To evaluate B-cell levels in infants of lactating women with CIS or MS receiving ocrelizumab postpartum	• B-cell levels (CD19+ cells, absolute counts and percentage of lymphocytes) measured at Day 30 after the mother's first ocrelizumab postpartum infusion

Objectives	Corresponding Endpoints
Secondary Outcome Measures (cont.)	
• To evaluate transfer of ocrelizumab into breastmilk of lactating women with CIS or MS receiving ocrelizumab postpartum	• AUC of ocrelizumab in mature breastmilk (i.e., milk produced after Day 14 postpartum) over 60 days after the first postpartum ocrelizumab infusion using the following time points: – If receiving 1 × 600 mg: before infusion and at 24 hours (Day 1), Day 7, Day 30 and Day 60 post-infusion – If receiving 2 × 300 mg: before infusion 1 and at 24 hours (Day 1), Day 7, Day 14, Day 15 (24 hours after infusion 2), Day 21, Day 30, and Day 60 post-infusion 1 • Average and peak ocrelizumab milk concentration as well as time to reach peak milk concentration, measured over 60 days after the mother's first postpartum ocrelizumab infusion
• To evaluate the *relative and* maximum exposure to ocrelizumab in infants of lactating women with CIS or MS receiving ocrelizumab postpartum	• Estimated MDID calculated as the peak ocrelizumab milk concentration multiplied by an estimated infant milk intake of 150 mL/kg/day measured over 60 days after the mother's first postpartum ocrelizumab infusion • *Average RID over 60 days, calculated as the ADID (mg/kg/day) divided by the maternal dosage (mg/kg/day) multiplied by 100* *Note:* Other pharmacokinetic and exposure parameters may be calculated as appropriate, based on the data obtained
• To evaluate whether there is transfer of ocrelizumab from the mother to the infant via breastmilk	• Serum concentration of ocrelizumab in the infant measured at Day 30 after the mother's first ocrelizumab postpartum infusion
• To evaluate whether infants of lactating women with CIS or MS receiving ocrelizumab postpartum are able to mount humoral immune responses to clinically relevant vaccines	• Mean titers of antibody immune response(s) to common childhood vaccinations with full or partial doses given prior to 1 year, which include responses to MMR, diphtheria, tetanus, pertussis, Hib, HBV, and PCV-13 • Proportion of infants with positive humoral response (seroprotective titers; as defined for the individual vaccine) to vaccines
Safety Objectives	
• To evaluate the safety of ocrelizumab in lactating women with CIS or MS receiving ocrelizumab postpartum and in their respective infants	• Rate and nature of adverse events in the mother throughout the study, including changes in clinical and laboratory results • Rate and nature of adverse events in the infant throughout the study, including infections and hospitalizations

Objectives	Corresponding Endpoints
Exploratory Objectives	
• To evaluate infant's growth velocity and developmental milestones in the first year of life	• Assessment of growth velocity based on age-adjusted length, weight, head circumference, using monthly growth charts according to the WHO Child Growth Standards, as well as absolute values at Months 2, 4, 6, 9, and 12 • Assessment of child developmental milestones in the domains of communication, gross motor, fine motor, problem solving, and personal-social at Months 2, 4, 6, 9, and 12, using the ASQ-3
• To measure disease activity in lactating women with CIS or MS receiving ocrelizumab postpartum	• Number of MS relapses during the postpartum period (clinical relapses) • Mean change in the EDSS score from last pre-baseline measurement (up to 1 year before LMP) to baseline
• To evaluate the evolution of B-cell levels over the first year of life in infants of lactating women with CIS or MS receiving ocrelizumab postpartum	• Trajectory (absolute and percentage changes) of B-cells (CD19+ cells) *in the infant from* Day 30 after the mother's first ocrelizumab postpartum infusion *to* 1 month after the first or second dose of MMR vaccine, or Month 13 of age in case MMR vaccine is not planned to be administered

ADID = average oral daily infant dosage; ASQ-3 = Ages and Stages Questionnaire version 3; AUC = area under the milk concentration-time curve; CIS = clinically isolated syndrome; EDSS = Expanded Disability Status Scale; HBV = hepatitis B virus; Hib = Hemophilus influenzae type b; LLN = lower limit of normal; LMP = last menstrual period; MDID = maximum oral daily infant dosage; MMR = measles, mumps, and rubella MS = multiple sclerosis; PCV-13 = 13-pneumococcal conjugate vaccine; *RID = relative infant dose;* WHO = World Health Organization.

STUDY DESIGN

DESCRIPTION OF STUDY

This is a prospective, multicenter, open-label study in lactating women with CIS or MS (in line with the locally approved indications) who decided together with their treating physician to continue on, or start treatment with, OCREVUS™ (ocrelizumab) postpartum despite ocrelizumab currently not being recommended during lactation.

Note on referral to sites: Pregnant and lactating women with MS are often treated in a decentralized way between specialized and non-specialized centers. It is difficult to predict at which clinical sites eligible women will be identified; and activation of new sites that identify potential women is not viable since it could take several months, and would not be achieved in time to screen the women while they still meet the protocol inclusion criteria. By using established networks and pregnancy registries for referral, the study could be completed in a timely manner. For these reasons, women may be referred to study sites; and study visits may be home-based (conducted by a mobile nurse, and by the investigator using telemedicine [i.e., remotely]). Implementation of these elements will depend on local requirements as well as agreement by the investigator, and capacity to use telemedicine. The investigators will be informed about the approach that may be used in their country.

This study will enroll at least *10* women with CIS or MS who are breastfeeding or planning to breastfeed.

The study will consist of the following periods:

- Screening period: After providing written informed consent, women will enter a screening period for eligibility assessments. Considering that decisions on initiating or resuming treatment with a disease-modifying therapy (DMT) in the postpartum period are usually taken before or during pregnancy, screening may be conducted at any time from the third trimester until 24 weeks postpartum. Final inclusion will only take place for women who have delivered a healthy term infant and have made a decision to breastfeed their infant despite ongoing ocrelizumab treatment. General health and medical history of the infant will also be reviewed for eligibility.

  Women resuming treatment with ocrelizumab postpartum will be included only if the last exposure to ocrelizumab occurred more than 3 months before the last menstrual period (LMP) (i.e., women without potential fetal exposure) to exclude any interference between fetal exposure and exposure via lactation.

- Treatment and sampling period: Women fulfilling the inclusion/exclusion criteria will receive the ocrelizumab dose regimen as per the locally approved label. The first dose of ocrelizumab may be administered at any point between Week 2 and Week 24 postpartum, as an initial split dose of two 300 mg infusions (in 250 mL 0.9% sodium chloride) separated by 14 days or as a single 600 mg infusion (in 500 mL 0.9% sodium chloride) according to the local prescribing information. For women where a decision not to administer a second 300 mg infusion is taken after enrolment, continuation in the study will be allowed. *Dosing and treatment duration are at the discretion of the physicians, in accordance with local clinical practice and local labeling (U.S. Prescribing Information [USPI]; Summary of Product Characteristics [SmPC]).* If women did not experience a serious infusion-related reaction (IRR) with any previous ocrelizumab infusion, a shorter (2-hour) infusion can be administered for subsequent 600 mg doses (Summary of Product Characteristics [SmPC], United States Prescribing Information [USPI]). Women referred by healthcare professionals (HCPs) to participate in the trial may receive ocrelizumab treatment at their neurologist's site as part of their standard of care treatment.

  Maternal breastmilk samples will be collected over several time points up to 60 (± 2) days after the first postpartum ocrelizumab infusion at approximately the same time of day, although flexibility is allowed on collection timings to accommodate the mother and infant feeding schedule. The only exception is the first (Day 1) post-infusion breastmilk sample and, in women who received a 2×300 mg dose, the second (Day 15) post-infusion breastmilk sample, which should be collected 24 hours after the midpoint of the infusion. On days of collection, milk should be expressed from both breasts until completely emptied using an electric breast pump. The milk from each breast is then mixed and a sample (volume = 5 mL) is removed for analysis. The infant can be bottle-fed the remaining expressed milk. If the infant is not usually fed using a bottle, milk may be expressed from one breast only. If the mother presents with unilateral mastitis, milk should only be expressed from the unaffected breast, until the infection resolves. If mastitis presents bilaterally (rare), breastmilk collection should be stopped until the infection resolves.

  The infant blood sample will be collected at Day 30 (± 2 days) of lactation after the first ocrelizumab infusion administered postpartum, i.e., regardless of whether women receive a 600 mg or a 2×300 mg dose. Blood samples may be collected at home by a visiting nurse, or at the hospital as part of study visits. *Note:* If the infant's B-cell levels are found to be below lower limit of normal (LLN), repeat analyses may be done at unscheduled visits at the discretion of the investigator (in consultation with the Sponsor).

  A structured telephone interview will be conducted by site personnel every 2 weeks in the treatment and sampling period, for a general review, and to identify and collect information on any changes in the woman's and infant's health status (including the occurrence of MS relapses in the mother and use of and new concomitant medications) and possible adverse events in both the woman and the infant (particularly infections); women will also be asked if the ASQ-3 form is being filled out. No telephone contact is needed in weeks where the woman is performing on-site visits.

- Vaccination period: After the 60-day (±2 days) treatment and sampling period, infants will continue to be followed-up for growth (age-adjusted length, weight, head circumference) and developmental milestones up to 12 months of age. Growth charts (following the World Health Organization Child Growth Standards; WHO 2022), absolute values and the Ages and Stages Questionnaire, version 3 (ASQ-3) will be used; other standard measurements recorded by e.g., the pediatrician as part of routine post-natal care, may also be used.

  Infant laboratory assessments will be performed 1 month (+30 days) after the first or second dose of measles, mumps, and rubella (MMR) vaccine, or at Month 13 of age (+30 days), in case MMR vaccine is not planned to be administered, to evaluate whether infants are able to mount humoral immune responses to clinically relevant vaccines, and for measurement of B-cell levels. In case the mother decides to switch to another DMT or to stop DMT after the 60-day treatment and sampling period, the infant blood sample will still be collected.

  A structured telephone interview will be conducted by site personnel postpartum every 3 months in the vaccination period (in-between ocrelizumab infusions) for a general review, and to identify and collect information on any changes in the woman's and infant's health status (including the occurrence of MS relapses in the mother and use of and new concomitant medications) and possible adverse events in both the woman and the infant (particularly infections); women will also be asked if the ASQ-3 form is being filled out. No telephone contact is needed in weeks where the woman is performing on-site visit.
- Discontinuation: Women who decide to discontinue the study (this includes discontinuation of either the mother or the infant; this does not apply to treatment discontinuation) will be invited to attend an early study discontinuation visit (which may be conducted remotely, i.e., virtually or by telephone) as soon as possible. Depending on the timing of discontinuation, the following is recommended:

  Discontinuation before the infant blood draw at 30 (±2) days post-infusion 1: Collection of infant outcomes in the first year of life as per standard pharmacovigilance procedures

  - If the mother remains on treatment with ocrelizumab and decides to stop participating at 30 (±2) days after the first postpartum ocrelizumab infusion, attempts to collect the infant sample at 30 (±2) days should be made before discontinuation.
  - If the mother switches to another DMT, the infant sample at 30 (±2) days should not be collected.

  Discontinuation after the infant blood draw at 30 (±2) days post-infusion 1: Collection of infant outcomes in the first year of life as per standard pharmacovigilance procedures.

NUMBER OF WOMEN

This study will enroll at least *10* women with CIS or MS (in line with the locally approved indications) who are breastfeeding or planning to breastfeed.

END OF STUDY

The end of the study is defined as the date of the last assessment (vaccine response titers measured 1 month [+30 days] after the first or second dose of MMR vaccine, or at Month 13 of age (+30 days) if MMR vaccine is not planned to be administered) for the last infant. The primary analysis will be conducted at the end of the Treatment and Sampling Period (Day 60 [±2 days]).

LENGTH OF STUDY

The total length of the study, from screening of the first woman to the end of the study, is expected to be approximately *3* years. This includes an enrolment period of approximately *21* months and a woman's participation period of 16 months.

TARGET POPULATION

INCLUSION CRITERIA

The following criteria must be met for study entry:

- An Informed Consent Form (ICF) for participation of the maternal subject and her infant (for collection of blood, infant demographic and adverse event data) is signed and dated by the subject. Where applicable, the written ICF with respect to the infant is also signed and dated by the holder of parental rights as designated by the maternal subject
- Woman is able and willing to comply with the study protocol, according to the judgment of the investigator, in particular:
  - Woman is willing to breastfeed (either exclusively, or with formula supplementation) for at least 60 days after the first postpartum ocrelizumab infusion (this decision is to be taken prior to and independent from study participation)
  - Woman is willing to provide breastmilk samples before and after their first and, if applicable, second postpartum ocrelizumab infusion

    Note: Exposure to ocrelizumab includes administration of an initial split dose of two 300 mg infusions (in 250 mL 0.9% sodium chloride) separated by 14 days for women initiating treatment with ocrelizumab, or a single 600 mg infusion (in 500 mL 0.9% sodium chloride) for women already on treatment with ocrelizumab.
- Woman is between 18 and 40 years of age at screening
- Woman has a diagnosis of MS or CIS (in line with the locally approved indications)
- Woman has delivered a healthy term singleton infant ($\geq$ 37 weeks gestation)
- Infant is between 2–24 weeks of age at the time of the mother's first postpartum dose of ocrelizumab
- For women who received commercial ocrelizumab (OCREVUS) before enrolment: documentation that last exposure to ocrelizumab occurred more than 3 months before the LMP (i.e., excluded a potential fetal exposure) and was given at the approved dose of 2×300 mg or 1×600 mg
- Woman agrees to use acceptable contraceptive methods or alternative methods during the study as described below and, if applicable, upon study treatment discontinuation, as defined by the local prescribing information
  - The following contraceptive methods are considered acceptable (failure rate $>$ 1% [Clinical Trial Facilitation Group (CTFG)]): progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action; male or female condom with or without spermicide; cap, diaphragm, or sponge with spermicide; combination of male condom with cap, diaphragm, or sponge with spermicide (double-barrier method).

  Birth control methods that are highly effective (i.e., failure rate $<$ 1% [CTFG]) may also be used but are not required, and include: oral, intravaginal or transdermal combined hormonal contraception associated with inhibition of ovulation [unless not recommended or contraindicated during breastfeeding]; oral, injectable or implantable progestogen-only hormonal contraception associated with inhibition of ovulation; intrauterine device; intrauterine hormone-releasing system; bilateral tubal occlusion; vasectomized partner; sexual abstinence.

  Note: lactational amenorrhea method can be used to ensure adequate protection from an unplanned pregnancy, and the following three criteria must be met: 1) amenorrhea; 2) fully or nearly fully breastfeeding (no interval of $>$ 4–6 hours between breastfeeds); and 3) $<$ 6 months postpartum. If any of the three listed criteria change at any stage during the study, an alternative or additional method of acceptable contraception is required.

EXCLUSION CRITERIA

Mothers/infants who meet any of the following criteria will be excluded from study entry:

Exclusions related to the mother

- Hypersensitivity to ocrelizumab or to any of its excipients
- Woman received last dose of ocrelizumab <3 months before the LMP or during pregnancy (i.e., there was a potential fetal exposure to ocrelizumab)
- Active infections (note: the woman may be included once the infection is treated and is resolved; women with bilateral mastitis infection should not have samples collected until the infection is completely resolved)
- Prior or current history of primary or secondary immunodeficiency, or woman in an otherwise severely immunocompromised state. Woman may be re-screened and included if condition resolves
- Woman with known active malignancies, or being actively monitored for recurrence of malignancy including solid tumors and hematological malignancies (except basal cell and in situ squamous cell carcinomas of the skin). Women with high risk of breast malignancies undergoing prophylactic treatment with drugs such as tamoxifen are excluded
- Woman has history of breast implants, breast augmentation, breast reduction surgery or mastectomy
- Woman has prior or current history of chronic alcohol abuse or drug abuse
- Woman has any medical, obstetrical or psychiatric condition that, in the opinion of the investigator, would compromise the woman's ability to participate in this study
- Treatment with a DMT for CIS or MS during pregnancy and/or first weeks postpartum, with the exception of formulations of interferon-beta, glatiramer acetate or pulsed corticosteroids
- Drugs known to transfer to the breastmilk and with established or potential deleterious effects for the infant, including but not limited to aspirin (risk of Reye's syndrome), tetracyclines or fluoroquinolones
- Treatment with any investigational agent within 6 months or five half-lives of the investigational drug (whichever is longer) prior to the LMP, unless the investigational agent is ocrelizumab administered >3 months prior to the LMP in the context of a study or registry sponsored by Roche

Exclusions related to the infant

- Infant is >24 weeks of age at the time of the mother's first postpartum dose of ocrelizumab
- Infant has any abnormality that may interfere with breastfeeding or milk absorption, including but not limited to cleft palate and/or lip, congenital diaphragmatic hernia and esophageal atresia
- Infant has an active infection. Infant may be included once the infection resolves
- Infant has any other medical condition or abnormality that, in the opinion of the investigator, could compromise the infant's ability to participate in this study, including interference with the interpretation of study results
- Infant has any other medical condition or abnormality that, in the opinion of the investigator, could compromise the infant's ability to participate in this study, including interference with the interpretation of study results
- Infant has at least one documented brief resolved unexplained event (BRUE), as defined by the 2016 Guidelines of the American Academy of Pediatrics

Exclusions related to laboratory findings

- Mother with any abnormal screening laboratory value that is clinically relevant should be retested only once in order to rule out any progressive or uncontrolled underlying condition. The last value before study entry must meet study criteria.

- Mother with positive screening tests for hepatitis B, determined by a positive hepatitis B surface antigen (HBsAg) result (current infection) or positive hepatitis B core antibody (HBcAb) titers (previous infection) will be excluded. Women with documented history of hepatitis B virus (HBV) vaccination or positive hepatitis B surface antibody (HBsAb) titers are eligible.

Note: based on local Ethics Committees (ECs) or National Competent Authority requirements, additional local diagnostic testing may be required for selected women or selected centers to exclude tuberculosis, Lyme disease, human T-lymphotropic virus 1 associated myelopathy (HAM), human immunodeficiency virus (HIV), hepatitis C virus infection (HCV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), hereditary disorders, connective tissue disorders, or sarcoidosis. Other specific diagnostic tests may be requested when deemed necessary by the investigator.

STUDY TREATMENT

The study treatment is commercial ocrelizumab.

PREMEDICATION

According to the label, 100 mg IV methylprednisolone (or an equivalent) and an antihistamine must be administered prior to administration of each ocrelizumab infusion to reduce the frequency and severity of IRRs. Premedication with an antipyretic (e.g., paracetamol) may also be considered prior to each ocrelizumab infusion

STATISTICAL METHODS

PRIMARY ANALYSIS

The primary analysis will be conducted on the full analysis set (all women who meet the eligibility criteria and received any postpartum dose of ocrelizumab) and infants of women in the full analysis set. The analysis will be performed after the last breastmilk sample collection at the end of the 60-day treatment and sampling period.

The proportion of infants with B-cell levels below the LLN will be calculated and the corresponding two-sided Clopper-Pearson 95% CI will be presented. The estimated average oral daily infant dosage will be analyzed using descriptive statistics. Mean, corresponding 95% CI, standard deviation, and other statistics will be presented.

More details about missing data handling, as well as sensitivity analyses based on alternative imputation approaches, will be specified in the statistical analysis plan.

DETERMINATION OF SAMPLE SIZE

The study will include at least 10 women with CIS or MS (in line with the locally approved indications) who are breastfeeding or planning to breastfeed.

With 10 infants, a precision (width of the two-sided 95% CI) of 0.443 is expected if one event is observed (defined as B-cells below the LLN) and a precision of 0.531 if two events are observed in the study. If no event is observed during the study, there is a 95% confidence that the event rate is below 0.31.

Appendix 2 Schedule of Assessments: Screening Through the End of Treatment Period

Visit [a]		Screening [b]	Treatment and Sampling Period [a]								Vaccination Period		Early Study Discontinuation Evaluation	
		1	2	3	4	2a [a]	3a [a]	4a [a]	5	6	–	7		
Day of visit		(Variable)	0 (baseline)	1	7	14	15	21	30 (± 2 days)	60 (± 2 days)	Months 2, 4, 6, 9, and 12	Month 13 of Age (+ 30 days) [c]	Woman	Child
Patient population and ICF	Diagnosis confirmation [d]	x												
	Informed consent [e]	x												
	Review inclusion/ exclusion criteria	x	x											
Maternal general medical history and demographics [f]	Demographics (age, ethnicity, level of education)	x												
	Clinically significant diseases and surgery/ procedures	x	x			x							x	
	Smoking history and alcohol intake	x												
	Vaccination history	x												
	Height	x												
	Weight	x	x			x								

Visit [a]		Screening [b]	Treatment and Sampling Period [a]								Vaccination Period		Early Study Discontinuation Evaluation	
		1	2	3	4	2a [a]	3a [a]	4a [a]	5	6	–	7		
Day of visit		(Variable)	0 (baseline)	1	7	14	15	21	30 (± 2 days)	60 (± 2 days)	Months 2, 4, 6, 9, and 12	Month 13 of Age (+ 30 days) [c]	Woman	Child
	Previous and concomitant medication	x	x			x							x	
Maternal MS disease history	Date of MS onset and diagnosis	x												
Maternal MS disease history (cont.)	Disease status (EDSS and relapses up to 1 year before the LMP)	x												
	History of previous DMTs (prior and/or during pregnancy)	x												
	Treatment history with ocrelizumab (OCREVUS) [g]	x												
Maternal obstetric history	Previous pregnancies	x												

Visit [a]		Screening [b]	Treatment and Sampling Period [a]								Vaccination Period		Early Study Discontinuation Evaluation	
		1	2	3	4	2a [a]	3a [a]	4a [a]	5	6	–	7		
Day of visit		(Variable)	0 (baseline)	1	7	14	15	21	30 (± 2 days)	60 (± 2 days)	Months 2, 4, 6, 9, and 12	Month 13 of Age (+ 30 days) [c]	Woman	Child
Maternal physical assessments	General physical examination h	x	x			x							x	
	Neurological examination i		x										x	
	Recording of potential relapses		x			x							x	
	EDSS score		x										x	
Maternal laboratory assessments	Breastmilk ocrelizumab concentration j		x	x	x	x	x	x	x	x				
	Hematology, chemistry, urinalysis [k]	x	x			x							x	
	Hepatitis B virus (HBV) screening [l]	x												
	Whole blood sample for lymphocyte subtypes [m]	x												

Visit [a]		Screening [b]	Treatment and Sampling Period [a]								Vaccination Period		Early Study Discontinuation Evaluation	
		1	2	3	4	2a [a]	3a [a]	4a [a]	5	6	–	7		
Day of visit		(Variable)	0 (baseline)	1	7	14	15	21	30 (± 2 days)	60 (± 2 days)	Months 2, 4, 6, 9, and 12	Month 13 of Age (+ 30 days) [c]	Woman	Child
	Serum Ig concentration	x												
Ocrelizumab infusion	Methyl-prednisolone and antihistamine premedication [n]		x			x								
	Ocrelizumab administration [o]		x			x								
	Documentation of collection of second postpartum ocrelizumab administration [ee]											x		
Infant physical assessments and procedures	General health and medical history [p]	x												
	Body weight for safety								x			x		
	Pregnancy and infant outcomes [q]	x												
	Feeding schedule diary and status [r]		x	x	x	x	x	x	x	x	x			

Visit [a]		Screening [b]	Treatment and Sampling Period [a]								Vaccination Period		Early Study Discontinuation Evaluation	
		1	2	3	4	2a [a]	3a [a]	4a [a]	5	6	–	7		
Day of visit		(Variable)	0 (baseline)	1	7	14	15	21	30 (± 2 days)	60 (± 2 days)	Months 2, 4, 6, 9, and 12	Month 13 of Age (+ 30 days) [c]	Woman	Child
	ASQ-3 [s]										x			x
	Documentation of infant growth velocity (weight, length, head circumference) [t]										x			x
	Previous and concomitant medications [u]	x							x		x	x		x
	Documentation of vaccination of the infant as part of routine care [v]										x	x		x
Infant laboratory assessments [w, x,y]	Whole blood sample for lymphocytes subtypes sample [y, z]								x			x		

Visit [a]		Screening [b]	Treatment and Sampling Period [a]							Vaccination Period		Early Study Discontinuation Evaluation	
		1	2	3	4	2a [a]	3a [a]	4a [a]	5	6	–	7	
Day of visit		(Variable)	0 (baseline)	1	7	14	15	21	30 (± 2 days)	60 (± 2 days)	Months 2, 4, 6, 9, and 12	Month 13 of Age (+ 30 days) [c]	Woman	Child
	Serum titers (IgG) of antibody immune responses to vaccinations [z, aa, bb]											x		
	Serum ocrelizumab concentration								x					
Telephone interview	General review of mother and infant [cc]			(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Safety	Adverse event assessment [dd]	x	x	x	x	x	x	x	x	x	x	x	x	x

Mother's assessments Infant's assessments

APGAR = appearance, pulse, grimace, activity, and respiration; ASQ-3 = Ages and Stages Questionnaire, version 3; BRUE = brief resolved unexplained event; CIS = Clinically isolated syndrome; DMT = Disease-modifying therapy; eCRF = electronic case report form; EDSS = Expanded Disability Status Scale; GGT = Gamma-glutamyl transpeptidase; HBcAb = Hepatitis B core antibody; HBsAb = Hepatitis B surface antibody; HBsAg = Hepatitis B surface antigen; HBV = Hepatitis B virus; HCPs = Healthcare professionals; Hib = *Hemophilus influenzae* type b; ICF = Informed Consent Form; *IRR = infusion-related reaction;* LLN = Lower limit of normal; *LMP = last menstrual period;* MMR = measles, mumps, and rubella; MS = multiple sclerosis; *NK = natural killer;* PCV-13 = 13-pneumococcal conjugate vaccine; *SmPC = Summary of Product Characteristics; USPI = U.S. Prescribing Information;* WHO = World Health Organization.

Note: 'x' indicates an assessment or procedure is to be done at that visit, and '(x)' indicates that depending on the situation, the assessment or procedure may or may not be done at that visit (e.g., the telephone interview will not be conducted in a week where there will be an on-site visit).

[a] Visits 2a, 3a and 4a are only applicable for women who will be receiving the first ocrelizumab dose as two 300 mg infusions.

[b] The length of the screening period is variable and depends on local timings for performing some of the eligibility assessments. It is possible that visit 1 (screening) is completed in one day or over several days/weeks. Screening may be started during the third trimester of pregnancy and continue until 24 weeks postpartum.

[c] Samples will be collected 1 month (+30 days) after the first dose of MMR vaccine (if first dose is administered at 11 months of age or later) or 1 month (+30 days) after second dose of MMR vaccine (if first dose is administered before 11 months of age), or at Month 13 of chronological age (+30 days) if MMR vaccine is not planned to be administered.

[d] The following diagnoses are accepted: MS or CIS (in line with the locally approved indications).

[e] Written informed consent will be obtained from all women at screening in order to be eligible for the study. Where applicable, the written ICF with respect to the infant is also signed and dated by the holder of parental rights as designated by the maternal subject.

[f] Medical history includes clinically significant diseases, surgeries/procedures, smoking history, alcohol intake and all medications (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, and nutritional supplements) used by the woman within 6 weeks prior to the baseline visit. Demographic data will include age, self-reported race/ethnicity, and level of education. Clinically significant diseases and/or surgeries and concomitant medication should also be recorded throughout the study. Information on vaccinations administered to the mother during the study will be collected under concomitant medications.

[g] Documentation of start of ocrelizumab (OCREVUS) therapy and date and dose of last ocrelizumab infusion prior to enrolment. Ocrelizumab-related information to be collected only in women who received commercial ocrelizumab prior to enrolment.

[h] A complete physical examination should be performed at the screening and baseline visits and at all visits during the treatment period (results from examinations done as part of routine care at subject's HCPs [obstetrician/gynecologist, pediatrician, neurologist of referred subjects] may be used). Any abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities not related to MS should be recorded as adverse events on the Adverse Event eCRF.

[i] Neurological examinations will be used to distinguish relapse in MS from another neurological (non-MS) disorder. Potential relapses should be recorded throughout the treatment period. *To reduce the burden of visits to mothers, results from neurological examinations done as part of*

routine care may be used. For patients referred to the investigator, results from routine visits at the woman's neurologist may be used.

[j] Women should record the volume of pumped breastmilk and indicate date and time for collection as well as whether breastmilk was pumped from one or both breasts. *For sampling on Day 0 (baseline), the sample should be taken before the infusion.* For 24 hours post-infusion (Day 1 and Day 15), samples are collected based on the midpoint of infusion. For example, if the infusion began at 8 am and ended at 10 a.m., the 24-hour sample collection would occur at 9 a.m. on the day after the infusion. With the exception of the 24-hour (Day 1; and [for women who received a 2×300 mg dose] Day 15) post-infusion breastmilk collection time points, flexibility is allowed on collection timing to accommodate the mother and the infant feeding schedule. If the mother presents with unilateral mastitis, milk should only be expressed from the unaffected breast, until the infection resolves. If mastitis presents bilaterally (rare), breastmilk collection should be stopped until the infection resolves.

[k] Hematology will include hemoglobin, hematocrit, RBCs, WBC absolute or/and differential count (neutrophils, eosinophils, lymphocytes, monocytes, basophils), and quantitative platelet count. Chemistry will include potassium, sodium, chloride, random glucose, AST, ALT, GGT, creatinine, total bilirubin. Urine dipstick (pH, specific gravity, glucose, protein, ketones, blood) and microscopic examination (sediment, RBCs, WBCs, casts, crystals, epithelial cells, bacteria) will be done at site locally at the discretion of the investigator.

[l] Women with positive screening tests for HBV, determined by a positive HBsAg result (current infection) or positive HBcAb titers (previous infection) will be excluded. Women with documented history of HBV vaccination or positive HBsAb titers are eligible.

[m] Blood samples will be collected to measure B-cell counts (CD19+ *and B-cell subsets [*Table 2 of the Study Protocol version 3]),T-cell counts (CD3+, CD4+, and CD8+*), and NK cell counts (CD16+CD56+).*

[n] All women must receive prophylactic treatment with 100 mg methylprednisolone (or an equivalent), administered by slow IV infusion, to be completed approximately 30 minutes prior to each ocrelizumab infusion and an antihistamine by oral or IV route, to be completed approximately 30–60 minutes prior to each infusion of ocrelizumab. The antihistamine should be the first premedication to be administered. The addition of an antipyretic (e.g., acetaminophen/paracetamol) may also be considered approximately 30–60 minutes prior to each infusion of ocrelizumab.

[o] In line with the dose regimen in the local label, the first dose of ocrelizumab may be administered as an initial split dose of two 300 mg infusions (in 250 mL 0.9% sodium chloride) separated by 14 days, or as a single 600 mg infusion (in 500 mL 0.9% sodium chloride), at any point between Week 2 and 24 postpartum. For women where a decision not to administer a second 300 mg infusion is taken after enrolment, continuation in the study will be allowed. *Dosing and treatment duration are at the discretion of the physicians, in accordance with local clinical practice and local labeling (USPI; SmPC).* If women did not experience a serious IRR with any previous ocrelizumab infusion, a shorter (2-hour) infusion can be administered for subsequent 600 mg doses. Women referred by HCPs to participate in the trial may receive ocrelizumab treatment at their neurologist's site as part of their standard of care treatment.

[p] General health and medical history for infants includes screening for the following (infants should be excluded from the study if any are present): age >24 weeks of age at the time of the mother's first postpartum dose of ocrelizumab; any abnormality that may interfere with breastfeeding or milk absorption, including but not limited to cleft palate and/or lip, congenital diaphragmatic hernia and esophageal atresia; an active infection (the infant may be included once the infection resolves); at least one documented BRUE, as defined by the 2016 Guidelines of the American Academy of Pediatrics.

[q] These will include: mode of delivery (vaginal delivery, instrumental delivery, scheduled or urgent cesarean section); APGAR score (1 min, 5 min, 10 min); gestational age at birth; infant's measurements (weight, length, head circumference); and congenital malformations.

[r] During the treatment and sampling period, women should record the number of breastmilk feeds and/or feeds with formula milk (supplementation) on the day of the sample collection and the previous and following day, i.e., day of collection ±1 day. During the vaccination period, women should record feeding status of the infant, i.e., whether exclusive breastfeeding, mixed feeding (partial breastfeeding along with infant formula and/or baby food), exclusive infant formula feeding, or fully weaned, at Months 2, 4, 6, 9, and 12, as applicable and depending on the infant's age at enrolment.

[s] Assessment of child developmental milestones in the domains of communication, gross motor, fine motor, problem solving, and personal-social will be captured at Months 2, 4, 6, 9, and 12 as applicable and depending on the infant's age at enrolment (see Appendix 6 of the Study Protocol version 3 for details of time windows of infant growth velocity and child developmental milestone assessments).

[t] Growth charts (according to the WHO Child Growth Standards; WHO 2022) will be used, as well as absolute values; other standard measurements recorded by e.g., the pediatrician as part of routine post-natal care may also be used). Infant growth will be captured at Months 2, 4, 6, 9, and 12 (see Appendix 6 of the Study Protocol version 3 for details of time windows of infant growth velocity and child developmental milestone assessments).

[u] Including documentation of past or current medications as well as clinically significant pediatric disease/abnormality. Changes to concomitant medication given to the infant should be recorded throughout the study.

[v] Documentation of vaccinations administered to the infant will be collected throughout the study.

[w] Infant sampling at Day 30 (±2 days) post-infusion 1 and at 1 month (+30 days) after first or second dose of MMR*, or* Month 13 of age *in case MMR vaccine is not planned to be administered* (+30 days) may be conducted via in-home nurse visits or at the hospital as part of study visits. CD19+ B-cell level at Day 30 (±2 days) post-infusion 1 represents the co-primary endpoint measurement.

[x] If the infant's B-cell levels are found to be below LLN, repeat analyses may be done at unscheduled visits at the discretion of the investigator (in consultation with the Sponsor).

[y] As per the recommendation of the EC ad hoc group (2008) the total blood volume to be collected from an infant in a clinical study should not exceed 0.8–0.9 mL/kg at any timepoint, or 2.4 mL/kg over any 4-week period throughout the study. If the blood volume collected for an infant (as a result of these limits) is insufficient to carry out all planned assessments, the order of priority for assessments is as follows: for sample at Day 30 (±2 days) post-infusion 1, (1) safety laboratory samples [scheduled or unscheduled and performed at the discretion of the investigator] (2) lymphocyte subtypes sample for B-cell counts (CD19+) and T-cell counts (CD3+, CD4+, and CD8+*), and NK cell counts (CD16+CD56+).* (3) serum ocrelizumab concentration; for the Month 13 of age/1 month after first/second MMR vaccine dose sample, (1) safety laboratory samples [scheduled or unscheduled and performed at the discretion of the investigator] (2) serum titers of antibody response to immunizations (3) lymphocyte subtypes sample for B-cell counts (CD19+) and T-cell counts (CD3+, CD4+, and CD8+*), and NK cell counts (CD16+CD56+).*

[z] For 1 month (+30 days) after the first or second dose of MMR vaccine, or at Month 13 of age (+30 days) if MMR vaccine is not planned to be administered, all efforts will be made to collect samples. However, if they cannot be collected, it will not be considered a protocol deviation.

[aa] Serum anti-vaccine antibody (IgG) titers will be measured to vaccines administered as per local practice over the first year of life (which include MMR, *diphtheria, tetanus, pertussis*, Hib, HBV and PCV-13); 1 month (+30 days) after the first dose of MMR vaccine (if first dose is administered at 11 months of age or later) or 1 month (+30 days) after second dose of MMR vaccine (if first dose is administered before 11 months of age), or at Month 13 of chronological age (+30 days) if MMR vaccine is not planned to be administered.

[bb] Vaccines administered from birth throughout the end of the study should be recorded at Months 2, 4, 6, 9, and 12 as well as at Month 13/1 month after first or second MMR dose.

[cc] A structured telephone interview will be conducted by site personnel postpartum every 2 weeks during the treatment and sampling period and every 3 months during the vaccination period (in-between ocrelizumab infusions) for a general review, and to identify and collect information on any changes in the woman's and infant's health status (including the occurrence of MS relapses in the mother and use of new concomitant medications) and possible adverse events in both the woman and the infant (particularly infections); women will also be asked if the ASQ-3 form is being filled out. No telephone contact is needed in weeks where the woman is performing on-site visits.

[dd] Adverse events in both mother and infant will be reported throughout the study as per standard pharmacovigilance procedures. Adverse events will also be captured at screening for women who received ocrelizumab before pregnancy.

[ee] *Documentation of premedication is not required.*

Appendix 3 B-Cell Reference Ranges by Week of Life: Absolute and Percentage Counts

TABLE III. B-cell reference ranges by week of life: Absolute and percentage counts

	Absolute B-cell count (cells/μL)			Percentage B-cell count (%)		
Week	Mean	LLN*	ULN†	Mean	LLN*	ULN†
1	452	127	1165	11.3	4.6	23.1
2	513	144	1322	12.1	5.0	24.9
3	577	163	1489	13.1	5.4	26.8
4	645	182	1664	14.0	5.8	28.7
5	716	202	1846	14.9	6.1	30.5
6	788	222	2033	15.8	6.5	32.4
7	863	243	2225	16.7	6.9	34.3
8	937	264	2418	17.6	7.2	36.1
9	1012	285	2612	18.4	7.6	37.8
10	1087	306	2803	19.3	7.9	39.5
11	1159	327	2991	20.0	8.3	41.1
12	1230	346	3172	20.8	8.6	42.7
13	1297	365	3346	21.5	8.9	44.1
14	1361	383	3511	22.2	9.1	45.5
15	1420	400	3665	22.8	9.4	46.8
16	1475	416	3807	23.4	9.6	47.9
17	1525	430	3935	23.9	9.8	49.0
18	1570	442	4050	24.3	10.0	49.9
19	1609	453	4151	24.7	10.2	50.7
20	1642	463	4237	25.1	10.3	51.5
21	1670	470	4308	25.4	10.5	52.1
22	1692	477	4364	25.6	10.6	52.5
23	1708	481	4406	25.8	10.6	52.9
24	1719	484	4435	25.9	10.7	53.2
25	1725	486	4450	26.0	10.7	53.4
26	1726	486	4453	26.1	10.7	53.5
27	1723	485	4445	26.1	10.8	53.5
28	1716	483	4426	26.1	10.7	53.5
29	1705	480	4398	26.0	10.7	53.3
30	1691	476	4362	25.9	10.7	53.2
31	1675	472	4319	25.8	10.6	52.9
32	1656	466	4270	25.6	10.6	52.6
33	1635	460	4216	25.5	10.5	52.3
34	1613	454	4159	25.3	10.4	51.9
35	1589	448	4099	25.1	10.3	51.5
36	1566	441	4037	24.9	10.3	51.0
37	1541	434	3975	24.7	10.2	50.6
38	1517	427	3912	24.4	10.1	50.1
39	1494	421	3851	24.2	10.0	49.7
40	1471	414	3792	24.0	9.9	49.2
41	1449	408	3735	23.8	9.8	48.8
42	1428	402	3682	23.6	9.7	48.4
43	1409	397	3632	23.4	9.6	48.0
44	1391	392	3588	23.2	9.6	47.6
45	1376	388	3548	23.0	9.5	47.3
46	1363	384	3515	22.9	9.4	47.0
47	1352	381	3488	22.8	9.4	46.7
48	1345	379	3468	22.7	9.3	46.5
49	1340	377	3456	22.6	9.3	46.3
50	1339	377	3452	22.5	9.3	46.2
51	1341	378	3458	22.5	9.3	46.2
52	1347	379	3475	22.5	9.3	46.3

LLN, Lower limit of normal; *ULN*, Upper limit of normal.
*Defined as the 2.5th percentile of B-cell count.
†Defined as the 97.5th percentile of B-cell count.

Extracted Borriello et al. 2022.

Appendix 4 Age-Specific Reference Ranges for Absolute Count (n × 10^6/L) of Lymphocyte Subsets

Subsets	Age group (months)	Lower Limit of Normal (90^{th} Percentile)
CD3+	0 to < 3	3180
	3 to < 12	2284
	12 to < 24	2542
CD4+	0 to < 3	2330
	3 to < 12	1523
	12 to < 24	1573
CD8+	0 to < 3	712
	3 to < 12	524
	12 to < 24	656
CD16+56	0 to < 3	201
	3 to < 12	230
	12 to < 24	186

Extracted from Tosato et. al. 2015.

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