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Clinical Development

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CBAF312ADE03 / NCT04792567

An open-label multicenter study to assess response to SARS-CoV-2 modRNA vaccines in participants with secondary progressive multiple sclerosis treated with Mayzent (siponimod) (AMA-VACC)

Statistical Analysis Plan (SAP)

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Document type:	SAP Documentation
Document status:	Final 3.0
Release date:	26-SEP-2022
Number of pages:	16

Document History – Changes compared to previous final version of SAP

Date	Time point	Reason for update	Outcome for update	Section and title impacted (Current)
12-APR-2021	Prior to FPFV	Creation of 1st draft version (0.1)	For Sponsor review	NA
20-APR-2021	Prior to FPFV	Creation of 2ndt draft version (0.2)	After Sponsor review	NA
29-APR-2021	Prior to first patient first vaccination	First final version	Finalization	NA
09-FEB-2022	After data review for 2nd interim analysis	CSP Amendment	Analyses of FACS data (CD4+ and CD8+ cell etc) added	
		Data Review	Subgroup analysis for patients with/without booster vaccination added	
26-SEP-2026	After final data review	Modification of Efficacy Analysis Set	To avoid that patients with non-missing secondary efficacy parameters are excluded from efficacy analysi set	

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List of abbreviations

AE	Adverse event
ATC	Anatomical Therapeutic Classification
CI	Confidence interval
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CV	Coefficient of variation
eCRF	Electronic Case Report Form
DMT	Disease modifying therapy
MedDRA	Medical Dictionary for Drug Regulatory Affairs
MS	Multiple sclerosis
RAP	Report and Analysis Process
SAP	Statistical Analysis Plan
SOC	System Organ Class
SmPC	Summary of Procduc Characteristics
TFLs	Tables, Figures, Listings
WHO	World Health Organization

1 Introduction

This Statistical Analysis Plan (SAP) describes how the statistical analyses of this study will be implemented. Based on the tables/listings/figures (TFL) of resulting from this SAP the Clinical Study Report (CSR) will be written. The TFLs will be attached in section 14 of the CSR.

In addition this SAP describes which patient Data Listings to be attached in section 16.2.1 of the CSR will be generated.

This SAP is not used for other analyses or studies.

It is based on the Study Protocol (CSP), version 02, dated 25th Sep 2021 (2nd Amendment)

1.1 Study design

This is a three-cohort, multicenter, open-label, prospective study of 60 patients suffering from multiple sclerosis (MS) currently treated with siponimod or a first-line disease modifying drug (DMT) or without MS treatment planning to undergo a SARS-CoV-2 modRNA vaccination as part of clinical routine and according to the respective SmPC.

MS treatments as part of clinical routine:

- Cohort 1: Siponimod treatment according to EU SmPC without interrupting daily dosing for the purpose of vaccination with SARS-CoV-2 modRNA
- Cohort 2: Siponimod treatment according to EU SmPC interrupting daily dosing for the purpose of vaccination with SARS-CoV-2 modRNA
- Cohort 3: Dimethylfumarate, glatirameracetate, interferon, teriflunomode as per respective EU SmPC or no current treatment with diagnosis of SPMS or with RRMS at risk to develop SPMS (at the discretion of the treating physician)

An interim analysis is planned after all participants have completed the study at one week after the end of the SARS-CoV-2 vaccination cycle in the study. The week +1 data regarding antibody and T-cell response (along with relevant safety data) will be examined as a preliminary evaluation of proof of concept and are the basis for the primary endpoint of the study.

The primary analysis time point is when all participants have completed the study visit 6 months after the end of the SARS-CoV-2 vaccination cycle in the study.

A follow-up analysis is planned after all participants have completed the follow-up visit 12 months.

1.2 Study objectives and endpoints

Objective(s)	Endpoint(s)
Primary Objective(s)	**Endpoint(s) for primary objective(s)**
• To estimate the proportion of those achieving seroconversion (i.e. having SARS-CoV-2 serum functional antibodies) after receiving a modRNA	Proportion of participants achieving seroconversion as defined by detection of SARS-CoV-2 serum functional antibodies one week after second dose of vaccine in participants treated

vaccine in participants treated concomitantly with siponimod and siponimod treatment break.	concomitantly with siponimod and siponimod treatment break (yes/no)
Secondary Objective(s)	**Endpoint(s) for secondary objective(s)**
• Describing SARS-CoV-2 serum functional antibody levels in participants treated concomitantly with siponimod versus siponimod treatment break versus first-line DMTs and in patients currently not on a DMT	• SARS-CoV-2 serum functional antibody levels one week, one month and six months after second dose of vaccine in participants treated concomitantly with siponimod versus siponimod treatment break versus first-line DMTs and in patients currently not on a DMT
• Describing the T-cell response to modRNA vaccines in participants treated concomitantly with siponimod versus siponimod treatment break versus first-line DMTs and in patients currently not on a DMT	• SARS-CoV-2 specific T-cell levels one week, one month and six months after second dose of vaccine in participants treated concomitantly with siponimod versus siponimod treatment break versus first-line DMTs and in patients currently not on a DMT measured by e.g. enzyme-linked immunosorbent spot (ELIspot) assay from peripheral blood mononuclear cells that were stimulated with SARS-CoV-2 peptide mix
• Describing safety, incl. AEs related to discontinuation and new onset of siponimod treatment and patients developing COVID-19	• AEs, SAEs, incl. patients with clinical confirmed COVID-19, events leading to discontinuation in participants treated concomitantly with siponimod versus siponimod treatment break versus first-line DMTs and in patients currently not on a DMT.

2 Statistical methods

2.1 Data analysis general information

Data analysis will be performed by the CRO [redacted]. The software SAS, version 9.2 or higher is used.

According to the small sample size of this study, percentages are presented without decimal places, i.e. as integers without digits.

2.1.1 General definitions

Study treatments

The modRNA vaccine and the treatment for MS are defined both as study treatments. More specifically, the MS treatment is established as study treatment from the time of informed consent.

Screening failure

A screening failure is a patient not eligible for the study. Screening failures are identified by:
- any inclusion criterion is violated or
  - Any exclusion criterion applies or
  - Patient was not vaccinated with a mRNA-vaccine or different mRNA-vaccines.

2.2 Analysis sets

For all analysis sets, participants will be analyzed according to the study treatment(s) received.

The safety analysis set (SAF) will include all participants that received any study drug.

The efficacy analysis set will include all participants who have a valid primary endpoint (i. e. determination of functional antibodies to SARS-CoV-2 at Visit 2 / Week 1) plus those who failed to receive their second dose of vaccine for whatever reason[1].

2.2.1 Subgroup of interest

Not applicable, no subgroup will be investigated.

2.3 Patient disposition, demographics and other baseline characteristics

Demographic and other baseline data including disease characteristics will be summarized descriptively by treatment group (Cohort 1-3) for the SAF and efficacy analysis set at Week 1.

If major imbalance in relevant demographic or other baseline charateristics are discovered, the study results will be interpreted with more caution and post-hoc supportive analyses may be conducted to adjust for the imbalance.

2.3.1 Patient disposition

Patient disposition displays the absolute and relative frequency of screening failures, the number of patients included in the different analysis sets.

The reasons for screening failure, for missed study visits, for exclusion from analysis populations and for prematurely drop-out are described by frequency tables as well as protocol deviations.

The duration of intervals between study visits are displayed by sample statistics. The interval from screening to first vaccination is analyzed by a frequency table for ≤30 days versus >30 days.

[1] Not applicable, because all patients received their second dose of study vaccine

2.4 Treatments (study treatment, rescue medication, concomitant therapies, compliance)

2.4.1 Study treatment / compliance

The absolute and relative frequency of performed study visits and performed vaccinations are presented. Sample statistics for the duration of intervals between screening and date of 1st vaccination, date of 1st and 2nd vaccination and date 2nd vaccination to Visit 1/Week 1 are displayed. The interval from the 2nd Vaccination to Visit 1/Week 1, Visit2 /Month 1 and Visit 3/Month 6 are also displayed by sample statistics. Here always the exact date of the respective vaccination is used but not the date of study visit called “Vaccination 1” or “Vaccination 2” which displays the date of phone contact with the patient.

Two types of investigational study drugs are used in this study:

1. Study medication for treatment of MS
2. Study medication for vaccination

The study medication for treatment of MS is displayed by frequencies for type of medication (cohort 3 only), sample statistics for duration of treatment prior to screening (cohort 1 and 3) and frequencies of dose changes, interruptions, or discontinuation until Visit 1, 2, and 3 respectively (cohort1 and 3). For cohort 2 the duration of siponimod interruption (i. e. the time between last dose prior to vaccination and the fist dose after vaccination) is displayed by sample statistics.

The different vaccines are displayed by a frequency table separately for 1st and 2nd vaccination.

2.4.2 Prior, concomitant and post therapies

The number and types of all pretreatments for MS prior to screening are displayed. Here, the current MS-therapy (i. e. starting before screening and ongoing after screening) is not included into counts.

The last disease modifying drug before siponimod (cohort 1 and 2) or the last study medication of cohort 3 is displayed in a frequency table.

Concomitant medications are coded by WHO Drug-dictionary. Frequencies of Anatomic Therapeutical Chemical (ATC) categories and Preferred Names are presented. Here, a medication with multiple entries is counted only once in a ATC category respectively a preferred name category.

2.5 Analysis of the primary objective

2.5.1 Primary endpoint

The **primary clinical question** in this trial is: what is the proportion of SPMS patients treated with siponimod within the three cohorts which mount an immune response to a SARS-CoV-2 modRNA vaccine?

The **primary endpoint** of the study is the proportion of participants achieving seroconversion as defined by detection of SARS-CoV-2 serum functional antibodies one week after second dose of

vaccine in participants treated concomitantly with siponimod and siponimod treatment break (yes/no).

Of note, this is not a randomized trial, therefore no randomization needs to be preserved and the ITT-principle and the associated estimands framework do not apply. However, this protocol will keep as close as appropriate to the terminology and the definitions established within that framework.
The justification for the primary estimand is that it will capture whether siponimod treated participants generate functional antibodies to SARS-CoV-2 vaccine as assessed by geometric mean titers and arithmetic mean for percent inhibition (semiquantitative).
The following attributes describe the primary estimand:

- **Population:** MS patients divided in three cohorts, each receiving a SARS-CoV-2 vaccination as part of clinical routine: (1) vaccination during continued siponimod treatment; (2) vaccination during a siponimod treatment interruption (of approx. 2-3 months); (3) vaccination in MS patients during continued treatment with first-line DMTs or no DMT. Patients who fail to receive their second dose of vaccine for whatever reason will be included in the analysis as non-responders. Patients who did receive their second dose of vaccine and do not have a valid determination of functional antibodies to SARS-CoV-2 for whatever reason will be excluded from the analysis of vaccine efficacy.
- **Variable:** Receiving the second dose of vaccine and achieving seroconversion as defined by detection of SARS-CoV-2 functional antibodies one week after second dose of vaccine (yes/no)
- **Treatment of interest:** siponimod either continuously applied or with a treatment break for the purpose of the vaccination, or first line DMTs or no treatment as part of clinical routine
- **Intercurrent events**. Failure to receive the second dose of vaccine: These patients will be counted as non-responders. Missing or invalid determination of functional antibodies to SARS-CoV-2: These patients will be excluded from the efficacy analyses (if they received their 2nd dose of vaccine)
- **Summary measure:** n.a., there will be no formal comparison between cohorts. Response rates will only be calculated within each cohort.

2.5.2 Statistical hypothesis, model, and method of analysis

The efficacy analysis set at Week 1 is used for all analyses of the primary parameter.

The primary analysis will not use any statistical testing or modelling. The absolute numbers and the proportion of participants achieving seroconversion within each cohort will be calculated. It will be augmented by a (descriptive) two-sided 95% confidence interval (exact Clopper-Pearson).

2.5.3 Handling of missing values/censoring/discontinuations

Patients who fail to receive their first dose of vaccine for whatever reason are excluded from the efficacy analysis Set(s). The number of these patients is displayed in the patient disposition table.

Patients who fail to receive their second dose of vaccine for whatever reason will be included in the analysis as non-responders. The number of these patients is displayed in the analysis table.

Patients who did receive their second dose of vaccine and do not have a valid determination of functional antibodies to SARS-CoV-2 for whatever reason will be excluded from the analysis of vaccine efficacy. The number of these patients is displayed in the analysis table.

2.5.4 Supportive analyses

If relevant differences in demographic or other baseline chracteristics are observed post-hoc analysis will be performed to adjust the primary endpoint for theses inhomogeneities. A logistic regression model would be applied.

The qualitative and (semi)quantitative antibody results towards coronaviridae are analyzed in addition. The first by frequency tables, the latter by sample statistics.

In several patients the booster vaccination was performed before Visit 3 at Months 6, so that the efficacy results were expected to be influenced by this booster vaccination. Therefore, the analyses of seroconversion in SARS-CoV-2 functional antibodies and reactivity to IFNg and IL-2 SARS-CoV-2 were presented for both subgroup of patient who received / did not receive a booster vaccination before Visit 3 at Month 3.

2.6 Analysis of the key secondary objective

Not applicable, because no key secondary objectives are specified.

2.7 Analysis of secondary efficacy objective(s)

2.7.1 Secondary endpoints

The secondary endpoints are analyzed descriptively within each cohort. The efficacy analysis set is used for all analyses of the secondary parameters.

The semiquantitative SARS-CoV-2 serum functional antibody titer (percent inhibitation) is summarized by arithmetic mean, standard deviation, minimum, median and maximum by visit. A boxplot graph will be presented.

The SARS-CoV-2 serum functional antibody titer is summarized by geometric mean, geometric coefficient of variation (CV) and 95% confidence intervals for the (geometric) mean. The Visits Screening, Visit 1/Week 1, Visit 2 /Month 1 and Visit3 /Month 6 are displayed separately in this summary table and a boxplot graph with logarithmic y-axis (log base 10).

The same summary table and boxplot will be provided for the following secondary parameters:

a) Qualitative and quantitative result of IFNg towards SARS-CoV-2 and towards coronaviridae

b) Qualitative and quantitative result of IL-2 towards SARS-CoV-2 and towards coronaviridae

c) Combined response in qualitative INFg or IL-s towards SARS-CoV-2 and towards coronaviridae, i. e. reactive to INFg or IL-2 or to both.

d) Cell viability, CD3 lymphocytes, CD4 and CD8 cells, percentage of CD4+ and CD8+ cell in peripheral blood mononuclear cells

The percent inhibition values for INFg at screening will be displayed for the subset of patients who are reactive at Week 1 versus non-reactive. The same analysis is provided for IL-2.

All other laboratory data (e. g. number of basal/background spots, spots, quality, comments, mutations) are listed only.

The MS activity is measured by relapses. The crude annual relapse rate until end of study and underlying sums (of relapses and patient-years) will be presented.

2.7.2 Statistical hypothesis, model, and method of analysis

Not applicable.

2.7.3 Handling of missing values/censoring/discontinuations

Missing values are not replaced.

2.8 Safety analyses

All safety analyses are presented for the Safety Set (SAF). Descriptive statistics are presented within study treatment groups (cohorts 1-3).

It should be mentioned that the follow-up analysis amends all analyses of adverse events. The CSR is planned to be finalized after this follow-up analysis.

2.8.1 Adverse events (AEs)

According to the CSP all adverse events starting in the on-treatment period, i. e. the treatment emergent AEs, are analyzed.

The on-treatment period lasts from the date of first administration of study treatment after informed consent to 30 days after the month 12 COVID-19 follow-up call.

The number (and percentage) of subjects with treatment-emergent adverse events will be summarized in the following ways:

- by treatment group (cohort 1-3), primary system organ class and preferred term.
- by treatment group (cohort 1-3), primary system organ class, preferred term and maximum severity

A subject with multiple AEs within a MedDRA primary system organ class is only counted once towards the total of the primary system organ class.

A subject with multiple AEs within a preferred term is only counted once towards the total of the preferred term class.

Separate summaries will be provided for study medication related AEs, death, SAEs, AEs leading to discontinuation and adverse events leading to dose adjustment.

The absolute and relative frequency of COVID-19 infections is presented.

Deaths and serious adverse events are listied in addition.

2.8.1.1 Adverse events of special interest / grouping of AEs

Adverse events related to COVID-19 are listed.

2.8.2 Deaths

Deaths are listed. A frequency table with absolute numbers, percentages and 95% confidence interval is presented.

2.8.3 Laboratory data

Not applicable.

2.8.4 Other safety data

2.8.4.1 Physical examination

Clinically significant findings which were not present at screening but at any later visits are displayed by a frequency table.

2.8.4.2 Vital signs

The changes from screening to Visit 1/Week 1, to Visit 2/Month 1, to Visit 3/Months 6 as well as differences between last and first value are displayed for pulse, systolic/diastolic blood pressure and body weight.

2.9 Pharmacokinetic endpoints

Not applicable.

2.10 PD and PK/PD analyses

Not applicable.

2.11 Patient-reported outcomes

Not applicable.

2.12 Biomarkers

Not applicable.

2.13 Other Exploratory analyses

Not applicable.

2.14 Interim analysis

See section 1.1 Study design.

3 Sample size calculation

The sample size of 20 participants per arm is selected based on need for early availability of results for the current covid-19 pandemic and the feasibility to recruit sufficient participants from AMASIA and PANGAEA 2.0 EVOLUTION. This sample size of 20 subjects will provide estimates of proportion responded with margin of error (half-width of a 95% confidence interval) of 20.1%, 19%, and 17.5% corresponding to response rates of 70%, 75%, and 80%, respectively. Adjusting for 10%

drop-out, 22 subjects will be enrolled in each arm. In case of fast recruitment the sponsor is allowed to increase sample size in each arm by up to 10 additional patients to support the generation of meaningful data in a larger sample size.

4 Change to protocol specified analyses

The definition for the Efficacy analysis Set was adapted to avoid that non-missing secondary endpoints would be excluded from the efficacy analysis set. For the rate of seroconversion (primary parameter) the patients with missing data are already excluded from the denominator.

5 Appendix

5.1 Imputation rules

No imputations will be done except:

If differences between dates are calculated, partial dates are replaced by 15/6 for days/month. If negative date differences result from this replacement, the value is set to 0.

5.2 Treatment-emergent adverse events

Treatment emergent AEs are those which start in the on-treatment period. The on-treatment period lasts from the date of first administration of study medication after informed consent to 30 days after the month 12 COVID-19 follow up call.

The date of first administration of study medication is derived as follows:

Cohort 1 (continued siponimod treatment): Because patients eligible for cohort 1 should have stable siponimod treatment before the study, the date of informed consent is used as date of first study medication. Deviations against this assumption will be detected during the data review meeting and monitoring of protocol deviations. If, necessary this derivation will be adapted.

Cohort 2 (interrupted siponimod treatment): Siponimod treatment can be interrupted before or after the informed consent. For patients who interrupted siponimod before informed consent, the date of first study medication is the first siponimod dose after informed consent or the date of first vaccination, whatever is earlier. For patients who interrupted siponimod after informed consent the date of informed consent is the date of first study medication.

Cohort 3: For patients who are treated with a first-line DMT the date of first study medication is derived like in cohort 1. For patients who do not receive any DMT the date of first study medication is the date of first vaccination.

If no month 12 COVID-19 follow-up call is available, the on-treatment period lasts to the date of last visit plus 30 days.

5.3 Statistical models

5.3.1 Primary analysis

The primary parameter "seroconversion" is derived from the laboratory data as follows:

Seroconversion is achieved, when SARS-CoV-2 neutralizing antibodies were not found ("negative") at the time of screening but SARS-CoV-2 neutralizing antibodies are found ("positive") at visit 1, visit 2 respectively visit 3.

5.3.2 Key secondary analysis

Not applicable

5.4 Rule of exclusion criteria of analysis sets

Deviation ID	Description of Deviation	Exclusion in Analyses
I01	Incl. crit. no. 1 (IFC) violated	Excluded from all analysis Sets
O05	Primary parameter (SARS-2-CoV-2 antibodies at Week 1) is missing	Excluded from denominator to calculate the rate of seroconversion

Table 2 Subject Classification

Analysis Set	PD ID that cause subjects to be excluded	Non-PD criteria that cause subjects to be excluded
all	NA	Not having informed consent;
SAF/Efficacy Analysis Set	NA	Not having any study treatment
Efficacy Analysis Set(s)	NA	Vaccination 1 not done or Vaccination not done with mRNA or Different vaccines at first and 2nd vaccination or Positive neutralizing SARS-Cov-2 antibodies at screening

6 Reference

None.