Statistical Analysis Plan

NCT Number: NCT07052682

Title: A Multicenter, Single-arm, Open-label, Phase 1b Study to Explore the Mechanism of Action and Evaluate the Safety of Ontamalimab in Participants With Nonalcoholic Steatohepatitis With Fibrosis Stage 1 Through 4

Study Number: TAK-647-1001

Document Version and Date: Version 2.0, 18 Sep 2024

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STATISTICAL ANALYSIS PLAN

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Phase: **1b**

Version: **2.0**

Date: **18-SEP-2024**

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Based on:

Protocol Version: **Protocol Amendment 3**

Protocol Date: **18-SEP-2023**

REVISION HISTORY

Version	Approval Date	Primary Rationale for Revision
1.0		Not Applicable
2.0		Incorporate changes from Protocol Amendment 3. Removed liver biopsy, HepQuant, and MAdCAM1 assessments at the end of treatment.

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ABBREVIATIONS

ADA	antidrug antibody
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AR1	homogeneous first order autoregressive
ARH1	heterogeneous first order autoregressive
AST	aspartate aminotransferase
ATC	anatomical therapeutic class
BMI	body mass index
CCR9	C-C chemokine receptor type 9
CI	confidence interval
CRN	Clinical Research Network
CS	compound symmetry
CSH	heterogenous compound symmetry
CSR	clinical study report
cT1	iron-corrected T1 mapping
cT1 MRI	iron-corrected T1 mapping by magnetic resonance imaging
CXCR3	C-X-C motif chemokine receptor 3
DSI	disease severity index
ECG	Electrocardiogram
eCRF	electronic case report form(s)
ELF	Enhanced Liver Fibrosis
F	fibrosis stage
F2	fibrosis stage 2
F3	fibrosis stage 3
F4cc	fibrosis stage 4 compensated cirrhotic
FAS	Full Analysis Set
FAST	FibroScan-aspartate aminotransferase

FIB-4	Fibrosis-4 Index
GGT	gamma-glutamyl transferase
GLM	generalized linear model
hsCRP	high-sensitivity C-reactive protein
IL	Interleukin
INR	international normalized ratio
LLN	lower limit of normal
LS Means	least-square means
LSM	liver stiffness measurement
MAdCAM-1	mucosal addressin cell adhesion molecule-1
MedDRA	Medical Dictionary for Drug Regulatory Activities
MELD	Model for End-stage Liver Disease
MMRM	mixed effects model for repeated measure
MRI	magnetic resonance imaging
NAFLD	nonalcoholic fatty liver disease
NAS	Nonalcoholic Fatty Liver Disease Activity Score
NASH	nonalcoholic steatohepatitis
PDFF	proton density fat fraction
PK	pharmacokinetic(s)
PML	progressive multifocal leukoencephalopathy
PPAS	Per-Protocol Analysis Set
Pro-C3	neoepitope-specific N-terminal propeptide of type III collagen
PT	preferred term
Q4W	every 4 weeks
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous(ly)
SD	standard deviation
SOC	system organ class

T2DM	type 2 diabetes mellitus
TBL	total bilirubin
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
US	United States
WHO	World Health Organization

1.0 OBJECTIVES, ENDPOINTS AND ESTIMANDS

1.1 Objectives

1.1.1 Primary Objective

To evaluate safety and tolerability of ontamalimab 75 mg administered every 4 weeks (Q4W) in participants with nonalcoholic steatohepatitis (NASH) (defined as Nonalcoholic Fatty Liver Disease [NAFLD] Activity Score [NAS] ⩾3 with at least 1 point in lobular inflammation) with different fibrosis stages (NASH Clinical Research Network [CRN] F1 through F4cc participants).

1.1.2 Secondary Objective

To determine if the changes from baseline in key biomarkers (neoepitope-specific N-terminal pro-peptide of type III collagen [Pro-C3] and Enhanced Liver Fibrosis test [ELF]) and in cT1 MRI (iron-corrected T1 mapping by magnetic resonance imaging) provide a signal of a potential role of the MAdCAM-1 pathway in participants with NASH (defined as NAS ⩾3 with at least 1 point in lobular inflammation) with different fibrosis stages (NASH CRN F1 through F4cc participants) after 24 weeks of administration of ontamalimab 75 mg Q4W.

1.1.3 Exploratory Objectives

- *To evaluate the changes from baseline in inflammatory and cell trafficking markers in blood samples after 24 weeks of administration of ontamalimab 75 mg Q4W in participants with NASH (defined as NAS ≥3 with at least 1 point in lobular inflammation) with different fibrosis stages (NASH CRN F1 through F4cc participants) and overall.*
- *To evaluate the changes in fibrosis markers from baseline after 24 weeks of administration of ontamalimab 75 mg Q4W in participants with NASH (defined as NAS ≥3 with at least 1 point in lobular inflammation) with different fibrosis stages (NASH CRN F1 through F4cc participants) and overall.*
- *To evaluate the changes in liver fat content from baseline after 24 weeks of administration of ontamalimab 75 mg Q4W in participants with NASH (defined as NAS ≥3 with at least 1 point in lobular inflammation) with different fibrosis stages (NASH CRN F1 through F4cc participants) and overall.*
- *To evaluate the changes in spleen cT1 MRI from baseline after 24 weeks of administration of ontamalimab 75 mg Q4W in participants with NASH (defined as NAS ≥3 with at least 1 point in lobular inflammation) with different fibrosis stages (NASH CRN F1 through F4cc participants) and overall.*
- *To evaluate the changes from baseline in biochemistry after 24 weeks of administration of ontamalimab 75 mg Q4W in participants with NASH (defined as NAS ≥3 with at least 1 point in lobular inflammation) with different fibrosis stages (NASH CRN F1 through F4cc participants) and overall.*

- *To evaluate the pharmacokinetics (PK) of ontamalimab 75 mg Q4W in participants with NASH (NAS ⩾3 with at least 1 point in lobular inflammation) (NASH CRN F1 through F4cc participants).*
- *To evaluate immunogenicity of ontamalimab 75 mg Q4W in participants with NASH (NAS ⩾ 3 with at least 1 point in lobular inflammation) (NASH CRN F1 through F4cc participants).*

1.2 Endpoints

1.2.1 Primary Endpoints

- *Incidence of treatment-emergent adverse events (TEAEs)*
- *Number of participants with clinically significant changes in the following parameters from baseline to the end of the follow-up period (Week 36):*
    - *Laboratory tests*
    - *Electrocardiograms (ECGs)*
    - *Vital signs*
    - *Body weight*

1.2.2 Secondary Endpoints

- *Percent change from baseline in Pro-C3 through Week 24*
- *Percent change from baseline in ELF through Week 24*
- *Change from baseline in liver cT1 MRI at Week 24*

1.2.3 Exploratory Endpoints

- *Change from baseline to Week 24 in each of the following biomarker readouts for each fibrosis stage group (fibrosis stage 1 through 4) and overall in all participants:*
    - *Serum or plasma biomarkers including but not limited to: high-sensitivity C-reactive protein (hsCRP) (serum), interleukin (IL)-8 (serum), and calprotectin (plasma)*
    - *T and immune cell trafficking in circulating whole blood samples, including but not limited to: Th17/Th1 vs. Th2, β_7^+ T cells, CCR9, and C-X-C motif chemokine receptor 3 (CXCR3)*
    - *Transcriptomics in liver biopsy and whole blood samples*
- *Change from baseline to Week 24 in each of the following biomarker readouts for each fibrosis stage group (fibrosis stage 1 through 4) and overall in all participants:*
    - *Liver stiffness measure (LSM) by FibroScan*
    - *Fibrosis-4 Index (FIB-4)*

  - *FibroScan-aspartate aminotransferase (FAST) score*
- *Change from baseline to Week 24 in proton density fat fraction (PDFF) as measured by MRI-PDFF, part of the Liver MultiScan® for each fibrosis stage group (fibrosis stage 1 through 4) and overall in all participants.*
- *Change from baseline to Week 24 in spleen cT1 MRI for each fibrosis stage group (fibrosis stage 1 through 4) and overall in all participants.*
- *Change from baseline to Week 24 in each of the following biomarker readouts for each fibrosis stage group (fibrosis stage 1 through 4) and overall in:*
  - *Alanine aminotransferase (ALT)*
  - *Aspartate aminotransferase (AST)*
  - *AST/ALT ratio*
  - *Alkaline phosphatase (ALP)*
  - *Gamma-glutamyl transferase (GGT)*
  - *Total bilirubin (TBL)*
  - *International normalized ratio (INR)*
  - *Albumin*
- *Serum concentrations of ontamalimab over the treatment period.*
- *Incidence of formation of antidrug antibodies (ADAs) through the end of the follow-up period (Week 36).*

1.3 Estimand(s)

Not applicable. This is a Phase 1b exploratory biomarker study.

2.0 STUDY DESIGN

This is a multicenter, single-arm, open-label, Phase 1b study to explore the role of MAdCAM-1 in NASH by administering a MAdCAM-1 inhibitor (ontamalimab) in participants with indication of NASH via biopsy (NAS ≥3 with at least 1 point in lobular inflammation) with different fibrosis stages (NASH CRN F1 through F4cc participants). The role of MAdCAM-1 will be evaluated by the changes in inflammatory and fibrosis biomarkers, liver histology and circulating inflammatory cells, and liver chemistry tests compared with baseline. MRI-derived cT1

(iron-corrected T1 mapping) will be used to evaluate fibroinflammatory changes in the liver. Safety and tolerability of ontamalimab 75 mg will be assessed.

Approximately 30 participants are planned to be enrolled to ensure that at least 18 participants (approximately 12 participants with F1-F3 and approximately 6 participants with F4cc) complete

the 24-week treatment period. Not more than four participants with F1 fibrosis will be enrolled in this study. Enrollment of F1, F2 and F3 participants at a 1:1:1 ratio will not be required. The study will be conducted at up to 20 sites in the US.

Participant eligibility with full inclusion/exclusion criteria are listed in the study protocol.

Pro-C3, ELF, and other exploratory markers will be measured at 2 screening visits and Day 1 predose measurement during treatment period to determine intra- participant variability. This will enable the correct interpretation of the posttreatment decrease in these biomarkers relative to the intra-participant variability at baseline.

After safety and tolerability have been established in the first four noncirrhotic (F1-F3) participants who have received at least 3 doses of ontamalimab 75 mg and have been monitored for at least 12 weeks after the first dose of study drug, eligible F4cc participants will be allowed to enroll into the study. The screening period may be extended for up to 24 weeks for participants with results consistent with F4 fibrosis (ie, compensated cirrhosis) after the first screening visit (ie, a combination of Fibrosis-4 Index [FIB-4] ≥3.48 and liver stiffness measurement (LSM) ≥20 kPa, or an Agile 4 score ≥0.57), or have a screening liver biopsy or a historical liver biopsy that confirms F4 NASH (NAS ≥3 with at least 1 point in lobular inflammation) with F4 fibrosis (ie, cirrhosis), until safety and tolerability data are evaluated in the first four noncirrhotic (F1-F3) participants.

All eligible participants (F1-F4) will enter a 24-week, single-arm, open-label treatment period with ontamalimab 75 mg. Ontamalimab will be administered subcutaneously (SC) Q4W starting on Day 1 and the last dose is administered at Week 20 (total treatment period is 24 Weeks), followed by a 12-week safety follow-up period. Liver biopsy samples will be collected during screening. Fibro-inflammation will be assessed by cT1 MRI, liver stiffness by FibroScan at the start of the treatment period and at Week 24. At visits Q4W between baseline and Week 24, assessments of selected biomarkers (including but not limited to Pro-C3, ELF, hsCRP, and calprotectin), liver chemistry tests, and specific safety data collection (laboratory, adverse events [AEs], neurological assessments) will be performed.

Liver stiffness measurement by FibroScan will also be performed at Week 12. Safety and tolerability data will be continuously monitored and will be evaluated by the internal safety review committee (independent from the study team).

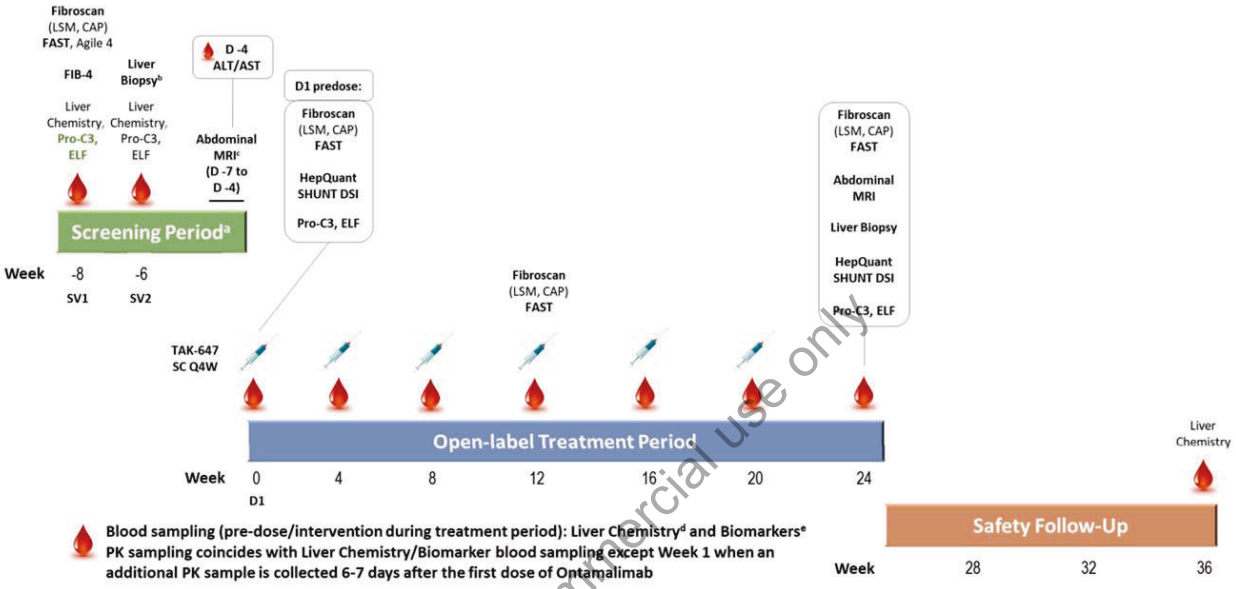
All participants, including those who discontinue, will undergo a safety follow-up period through 12 weeks after the last dose of study drug.

The end of the study is defined as the date of the last visit (Week 36) of the last participant undergoing the study, unless the study is stopped earlier by the sponsor due to futility or for safety reasons (Section 4.4 of the protocol).

2.1.1 Study Schedule

The study schema is presented below (Figure 2.a). Please refer to Section 1.3 of the protocol for detailed information pertaining to the schedule of assessments and procedures at each visit.

Figure 2.a Study Schema



[a] For participants with NASH (NAS ≥3) and F4cc fibrosis (ie, cirrhosis), the screening period may be extended up to 24 weeks until safety and tolerability information is available on first four noncirrhotic (F1-F3) participants who have received at least 3 doses of ontamalimab and have been monitored for at least 12 weeks after the first dose of study drug. Progression of screening is contingent on successful confirmation of eligibility along with the sponsor's approval.

[b] Eligibility for liver biopsy at Week -6 (SV2) will be established on the basis of results of Week -8 (SV1) assessments (including but not limited to Pro-C3 ≥12.6 ng/mL, ELF ≥7.7, and liver chemistry eligibility criteria as participants should meet all inclusion criteria and none of exclusion criteria for assessments and procedures at Week -8 (SV1) to be eligible for liver biopsy. For participants who meet other eligibility criteria at Week -8 (SV1), if ALT or AST is >4×ULN and <5×ULN at Week -8 (SV1), then the ALT/AST results at Week -6 (SV2) will also be evaluated before liver biopsy and the abdominal MRI can be performed. Only participants with indication of NASH via biopsy (NAS ≥3 with at least 1 point in lobular inflammation) and liver fibrosis stage F1 through F4cc will be enrolled in the study. Green fonts indicate key inclusion criteria. Qualifying historical liver biopsies are acceptable for screening purposes if they are available to be reviewed and read centrally by the study pathologists and if they meet all the criteria as defined in the histopathology manual. For participants with qualifying historical liver biopsies, the abdominal MRI visit (Visit 4a) may occur as soon as possible after liver chemistry results from SV2 become available, and SV2 and the Day -4 visit must be at least 2 weeks apart.

[c] Abdominal MRI may be performed between Day -7 and Day -4.

[d] Liver chemistry tests include ALT, AST, total bilirubin, alkaline phosphatase, gamma-glutamyl transferase, and albumin.

[e] Biomarkers from blood samples include serum Pro-C3; ELF; serum sMAdCAM-1; serum and plasma markers of inflammation, fibrosis, and trafficking (including but not limited to hsCRP, IL-8, and calprotectin); whole blood

T cell phenotyping (including but not limited to Th17/Th1 vs Th2 ratios, β_7^+ T cells, CCR9, and CXCR3) (treatment period only); and whole blood transcriptomics (treatment period only). Pro-C3 levels and ELF score at Week -8 (SV1) will be used to determine eligibility. Pro-C3 levels and ELF scores at Week -8 (SV1), Week -6 (SV2), and D1 predose measurement during treatment period will be used in calculating the baseline value for each parameter.

Footnote on End-of-Treatment Liver Biopsy, HepQuant, and MAdCAM1: The sponsor has removed the end-of treatment liver biopsy, HepQuant, and soluble MAdCAM-1 assessment from the study design due to the program's termination for strategic reasons.

3.0 STATISTICAL HYPOTHESES AND DECISION RULES

The statistical objective of this study is to estimate the effect of ontamalimab on biomarkers described in Sections 1.1.2 and 1.1.3. No formal hypothesis testing will be performed.

3.1 Statistical Hypotheses

Not applicable.

3.2 Statistical Decision Rules

Not applicable.

3.3 Multiplicity Adjustment

Not applicable.

4.0 SAMPLE-SIZE DETERMINATION

Approximately 30 participants are planned to be enrolled to ensure that at least 18 participants complete the 24-week treatment period. The sample size has been chosen to provide adequate number of participants to investigate the objectives of the study based on clinical experience for key biomarkers (Anstee et al. 2022; Harrison et al. 2018; Jabor et al. 2018).

This study is not statistically powered to perform any formal hypothesis testing, and the analyses will be based on estimation approach.

5.0 ANALYSIS SETS

5.1 Screened Analysis Set

The screened analysis set will consist of all participants who have signed informed consent(s).

5.2 All Enrolled Analysis Set

The all enrolled analysis set will consist of all participants who are enrolled (as defined in Section 5.6 of the protocol) in the study.

5.3 Full Analysis Set

The full analysis set (FAS) will consist of all participants who are enrolled in the study, have received at least 1 dose of study drug, and have at least 1 valid postbaseline assessment.

5.4 Per-Protocol Analysis Set

The per-protocol analysis set (PPAS) is a subset of the FAS and will include all participants who did not have any important protocol deviations affecting the efficacy evaluations. All decisions to exclude participants from the PPAS will be made before database lock. Analyses on the secondary endpoints using the PPAS will be provided as supplementary analysis.

5.5 Safety Analysis Set

The safety analysis set will consist of all participants who are enrolled in the study and have received at least 1 dose of study drug.

5.6 Pharmacokinetic Analysis Set

The PK analysis set will consist of all participants in the safety analysis set who have at least 1 evaluable postdose PK concentration value.

6.0 STATISTICAL ANALYSIS

6.1 General Considerations

6.1.1 Statistics and Precision

For continuous variables, descriptive statistics will include the number of participants (n), mean, standard deviation (SD), median, minimum, and maximum. The number of decimal places displayed for each statistic will be determined as follows:

- Mean and median: 1 more decimal place than the recorded data.
- SD: 2 more decimal places than the recorded data.
- Minimum and maximum: equal to the number of decimal places in the recorded data.
- Confidence intervals (CIs) will be presented using the same number of decimal places as the parameter estimate.

For categorical variables, the counts and proportions of each possible value will be presented. The denominator for the proportion will be based on the number of participants who provided non-missing responses to the categorical variable. Percentages will be reported to 1 decimal place.

6.1.2 Definition of Baseline

For biomarkers that are measured multiple times during screening, data from all available sampling (including the Day 1 predose measurement) will be averaged to establish the stable

baseline value. For all other biomarker and safety parameters, baseline values are defined as the last non-missing value collected before the first dose of study drug.

The change from baseline is calculated by subtracting the baseline values from the individual postbaseline values. If either the baseline or postbaseline value is missing, the change from baseline is set to missing.

The percent change from baseline is calculated by dividing the change from baseline values with the baseline values and multiplying by 100. If either the baseline or postbaseline values is missing, the percent change from baseline is set to missing.

6.1.3 Definition of Study Day and Visit Windows

A windowing convention will be used to determine the analysis value for a given study visit.

Study Day 1 is defined as the date on which a participant is administered their first dose of the study drug. Other study days are defined relative to Study Day 1. Relative day is calculated as (date of interest – date of first dose + 1) for study days on or after the first dose date, and as (date of interest – date of first dose) for study days prior to the first dose date.

For each visit, the analysis windows for biomarker and safety parameters are specified in Appendix A. More than one result for a parameter may be obtained in an analysis visit window. In such an event, the result with date closest to the scheduled visit day will be used. If two observations are equidistant to the scheduled visit day, the later of the observations will be used.

The analysis window convention will not be applied to the electronic case report form (eCRF) data listings. The data listings for eCRF data will display the raw data as collected and entered in the eCRF.

6.2 Disposition of Participants

A summary of screen failures by site and overall will be presented.

Disposition of all enrolled participants will be tabulated for the all enrolled analysis set. Categories will include:

- Participants who were enrolled but not treated.
- Participants who completed study drug.
- Participants who prematurely discontinued study drug and reason for discontinuation.
- Participants who completed the Week 24 visit.
- Participants who prematurely discontinued the study before Week 24 and reason for discontinuation.
- Participants who completed the study.
- Participants who prematurely discontinued the study and reason for discontinuation.

6.3 Demographic and Other Baseline Characteristics

Demographics and baseline characteristics will be summarized for the safety analysis set. Individual participant demographic and baseline characteristic data will be listed.

6.3.1 Demographics

Demographics summary will include descriptive statistics for age, height, weight, and body mass index (BMI). The number and percentage of participants within each category will be presented for sex, race (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian/Other Pacific Islander, White, and Not Reported), and ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not Reported, and Unknown).

6.3.2 Baseline Characteristics

The summary of baseline characteristics will include descriptive statistics for continuous variables and counts and percentages for categorical variables.

Table 5.a Baseline Characteristics

Baseline Characteristics	Summarized as	Categories
Fibrosis stage	Categorical	F1, F2, F3, F4cc
T2DM[a] at baseline	Categorical	Yes, No
Overall NAS[b] score	Continuous	
Assessment of lobular inflammation	Categorical	0, 1, 2, 3

[a] T2DM = type 2 diabetes mellitus
[b] NAS = Nonalcoholic fatty liver disease activity score

6.4 Medical History and Concurrent Medical Conditions

Medical history refers to significant conditions or diseases relevant to the disease under study that resolved before, or at the time when, the participant provided informed consent. Ongoing conditions are considered concurrent medical conditions.

Medical history and concurrent medical conditions will be coded using the latest version of Medical Dictionary for Drug Regulatory Activities (MedDRA) and will be summarized using system organ class (SOC) and MedDRA preferred term (PT) based on the safety analysis set. A participant will only be counted once within a particular MeDRA level even if they have multiple conditions/symptoms in that level.

All medical history and concurrent medical condition data will be listed by site (study center) and participant number.

6.5 Medication History and Concomitant Medications

Prior medications/treatments are defined as those that were received within 30 days of the date of first dose of study drug.

Concomitant medications/treatments are defined as those given in addition to the study drug between the dates of the first dose of study drug and the end of the follow-up period, inclusive.

In the case of missing or partial dates, the rules of imputation are defined in Appendix B.

Medication/treatment history and concomitant medications/treatments will be coded using the lates version of the World Health Organization (WHO) Drug Dictionary and summarized using PT within each anatomical therapeutic class (ATC) based on the safety analysis set. If a participant reports taking more than one drug belonging to the same WHO Drug level, they will only be counted once within that level.

6.6 Extent of Exposure and Compliance

The safety analysis set will be used for all summaries in this section. Study drug exposure, completed injections, and compliance will be summarized descriptively (n, mean, SD, median, minimum, maximum). The following parameters will be included:

- Duration of exposure (weeks) to study drug defined as (date of last dose – date of first dose +1)/7.
- Number and percentage of participants in the following duration category: ≤4 weeks, >4 and ≤8 weeks, >8 and ≤12 weeks, >12 and ≤16 weeks, >16 and ≤20 weeks, and >20 weeks.
- Total number of injections received.
- Overall compliance (%), calculated as the percentage of completed injections out of the total number of planned injections.
- Number and percentage of participants in the following overall compliance categories: <80%, and ≥80%.

All study drug administration data will be listed by site (study center) and participant number.

6.7 Biomarker and Noninvasive Test Analysis

The measurements of biomarkers and noninvasive tests collected at each scheduled visit will be summarized using descriptive statistics (n, mean, SD, median, minimum, and maximum). In addition, the change from baseline (including percent change from baseline for Pro-C3 and ELF) will be summarized descriptively for all postbaseline visits.

6.7.1 Secondary Endpoints Analysis

6.7.1.1 Main Analysis Approach

The secondary endpoint analysis for Pro-C3 and ELF, which are collected at multiple postbaseline visits, will be performed on the FAS. The percent change from baseline for these two biomarkers will be analyzed using a mixed effects model for repeated measure (MMRM) with visit as the fixed effect, participants as the random effect, and the baseline values as covariates. Least-square means (LS means) of the percent change from baseline at Week 24 along with the corresponding 95% CIs will be presented for Pro-C3 and ELF. Line chart of LS mean percent change from baseline over time obtained from the MMRM model may be displayed.

For the MMRM, an unstructured covariance structure will be used to model the within-participant errors. If the model fails to converge, the following changes will be applied in order:

- The model will be fitted using covariance matrices in the following order: heterogenous first order autoregressive (ARH1), homogenous first order autoregressive (AR1), heterogenous compound symmetry (CSH), and compound symmetry (CS).
- If the model still fails to converge, the percent change from baseline at Week 24 for Pro-C3 and ELF will be summarized descriptively.

The secondary endpoint analysis for liver cT1 MRI, which is collected at one postbaseline visit (Week 24), will be performed using participants from the FAS who have non-missing measurements of liver cT1 MRI at baseline and Week 24. No imputation will be carried out for missing measurements of liver cT1 MRI. The change from baseline at Week 24 for this biomarker will be summarized descriptively.

Distributional assumptions underlying the model will be assessed by visual inspection of residual plots. The assumption of normality will be assessed by examination of the normal probability plot. The assumption of homogeneity of variance will be assessed by plotting the residuals versus the predicted values from the model. If assumptions appear to be violated, the biomarker data at each visit will be summarized descriptively.

6.7.1.2 Supplementary Analysis

If the PPAS is different from the FAS, the main analysis approach for secondary endpoints described in Section 5.7.2.1 will be repeated on the PPAS to evaluate the robustness of key biomarker results.

6.7.2 Exploratory Endpoints Analysis

The exploratory endpoint analysis will be performed separately for each of the following biomarkers using participants from the FAS in fibrosis stages F1/F2/F3 and overall who have non-missing measurements of the biomarker at baseline and Week 24:

- Liver chemistry panel (ALT, AST, ALP, and TBL).

- Fibrosis markers (LSM, FIB-4, and FAST score).

The mean observed change from baseline at Week 24 will be summarized descriptively for fibrosis stages F1/F2/F3 and overall.

Exploratory biomarkers listed in Section 1.2.3 but not included in the exploratory endpoint analysis will not be included in the clinical study report (CSR) but analyzed and reported separately. The details of such analysis may be described in the Biomarker Analysis Plan as appropriate.

6.7.3 Subgroup Analyses

Subgroup analyses of all secondary endpoints will be performed on the FAS by fibrosis stage (F1/F2/F3 vs overall) and summarized descriptively. Tabulations and forest plots will be generated to display the results of each analysis.

6.8 Safety Analysis

All safety analysis will be performed using the safety analysis set.

Clinical laboratory test results, vital signs, body weight, and ECG findings will be summarized by visit. Potentially clinically significant findings will also be summarized or listed.

Antidrug antibody data will be summarized by visit.

6.8.1 Adverse Events

AEs will be coded using the MedDRA (version 25.0 or higher).

TEAEs are defined as AEs or serious adverse events (SAEs) that started or worsened in either intensity or frequency after receiving study drug (AE start date ≥ first dose date). Partial or fully missing AE dates will be imputed as defined in Appendix B.

TEAEs will be summarized by giving the number and percentage of participants reporting any event for each term. The following summaries will be presented:

- Overview of TEAEs.
- TEAEs by SOC and PT.
- TEAEs by severity, SOC, and PT.
- TEAEs by relationship to the study drug, SOC, and PT.
- Treatment-related TEAEs by SOC and PT.
- TEAEs leading to study drug discontinuation by SOC and PT.
- Treatment-related TEAEs leading to study drug discontinuation by SOC and PT.
- Treatment-emergent SAEs by SOC and PT.
- Treatment-emergent SAEs by severity, SOC, and PT.

- Treatment-emergent SAEs by relationship to study drug, SOC, and PT.
- Most frequent TEAEs (≥5%) by PT (in descending order)

SOCs will be sorted by alphabetical order. Within a SOC, PTs will be sorted in descending order based on the total number of participants with AEs. For each category and overall, participants reporting more than 1 occurrence for a term (SOC or PT) being summarized will be counted only once using the most extreme incident (most severe for intensity tables and related for the relationship to study drug tables). AEs with missing severity will be classified as having the highest severity and AEs with missing relationship will be classified as having the highest relationship to study drug.

Data listings will be provided for all TEAEs, treatment-related TEAEs, TEAEs leading to study drug discontinuation, SAEs, and AEs that resulted in death.

6.8.2 Adverse Events of Special Interest

An AESI (serious or nonserious) is one of scientific and medical concern specific to the compound or program, for which ongoing monitoring and rapid communication by the investigator to Takeda may be appropriate. AESIs will be reported by the investigators as part of the AE reporting process.

Adverse events of special interest in this study include potential hypersensitivity, serum sickness, vasculitis, Arthus reactions to ontamalimab, and PML. Participants will be monitored for the presence of Type I (anaphylaxis) and Type III (immune complex) hypersensitivity reactions at the time points specified in the Section 1.3 of the protocol. The following summaries of AESIs will be presented:

- Overview of AESIs.
- AESIs by SOC and PT.

A listing of all AESIs will be provided.

6.8.3 Clinical Laboratory Assessments

Clinical laboratory assessments that will be performed for the study are listed in Section 10.2 of the protocol.

The observed value and change from baseline in clinical laboratory assessments will be summarized descriptively by visit.

The number and percentage of participants with clinically significant laboratory values, as defined in Appendix C, will be summarized for each visit.

A listing of all laboratory data will be provided in standard units. Laboratory data outside of the normal reference range will be indicated in the listing.

6.8.4 Vital Signs and Weight

Vital sign measurements (body temperature [°C], respiratory rate [breaths/min], sitting blood pressure [mmHg], and pulse rate [beats/min]) and body weight (kg) will be collected before the collection of blood samples for laboratory assessments, where applicable.

The observed value and change from baseline in vital sign measurements and weight will be summarized descriptively by visit.

The number and percentage of participants with clinically significant vital sign and weight values, as defined in Appendix C, will be summarized for each visit.

A listing of all vital sign and weight data will be provided.

6.8.5 12-Lead ECGs

A local ECG reader will be used in this study. The investigator's ECG interpretation (i.e., within normal limits, abnormal but not clinically significant, abnormal and clinically significant, or not evaluable) will be summarized by visit.

A listing of all ECG data will be provided.

6.9 Pharmacokinetic Analyses

The PK analysis set will be used for all PK analyses.

PK samples will be collected at predose on Day 1 and predose at Weeks 4, 8, 12, 16, 20, and 24 during the treatment period, and approximately 1 week after the dose on Day 1. PK concentrations of ontamalimab over the sampling time will be descriptively summarized and graphed.

A listing of all PK concentrations will be provided.

6.10 Interim Analyses

No interim analysis is planned.

6.11 Other Committees

An internal group (independent from the study team) will review the overall safety of the study participants on an ongoing basis. This review will consist of monitoring of safety of participants throughout the study. Recommendations made on the basis of this review to alter the conduct of the study or amend the protocol will be provided to the study team for review and for a final decision. The sponsor or its designee will notify investigative sites, and regulatory authorities as appropriate, of recommendations based on this review. Details regarding this review and the frequency of this review will be established in a separate charter before the administration of study drug to any participant. In addition, this internal group will be responsible for:

- Evaluating safety and tolerability data from the first four noncirrhotic (F1-F3) participants who have received at least 3 doses of ontamalimab 75 mg and have been monitored for at least 12 weeks, to support the decision to include F4cc participants in the study.
- Engaging with an independent panel of experts to evaluate any liver-related events (eg, liver decompensation, significant deterioration of liver chemistry tests).
- Monitoring neurological safety and consulting a panel of leading progressive multifocal leukoencephalopathy (PML) experts, including a neurologist, neuroradiologist, and a virologist, in the event of a suspected case of PML (see Section 8.3.2 of the protocol).

6.12 Changes to Protocol Planned Analysis

The change from baseline at Week 24, in fibrosis stages F1/F2/F3 and overall, for cT1 MRI and exploratory endpoints will be summarized descriptively instead of analyzed using MMRM. This was simplified to fit the exploratory nature of these endpoints.

7.0 REFERENCES

1. Anstee, Q. M., Hallsworth, K., Lynch, N., Hauvespre, A., Mansour, E., Kozma, S., et al. 2022. Real-world management of non-alcoholic steatohepatitis differs from clinical practice guideline recommendations and across regions. JHEP Rep, 4(1), 100411.
2. Harrison, S. A., Dennis, A., Fiore, M. M., Kelly, M. D., Kelly, C. J., Paredes, A. H., et al. 2018. Utility and variability of three non-invasive liver fibrosis imaging modalities to evaluate efficacy of GR-MD-02 in participants with NASH and bridging fibrosis during a phase-2 randomized clinical trial. PLoS One, 13(9), e0203054.
3. Jabor, A., Kubíček, Z., Fraňková, S., Šenkeříková, R. and Franeková, J. 2018. Enhanced liver fibrosis (ELF) score: Reference ranges, biological variation in healthy participants, and analytical considerations. Clin Chim Acta, 483, 291-5.

8.0 APPENDIX

8.1 Changes From the Previous Version of the Statistical Analysis Plan (SAP)

- Removed liver biopsy, HepQuant, and MAdCAM1 assessments at the end of treatment and associated exploratory objectives/endpoints.
- Changed subgroup analyses for secondary and exploratory endpoints to F1/F2/F3 vs overall to prevent lack of model convergence due to small subgroups.
- Added additional AESI to match Protocol Amendment 3.

8.2 Appendix A - Definition of Analysis Visit Windows

Table 7.2.a Analysis Visit Window for Key Biomarkers

Visit	Scheduled Day	Pro-C3	ELF	Abdominal MRI
Baseline (Part 1)	Day -7	NA	NA	≤1
Baseline (Part 2)	Day 1	1	1	NA
Week 1	Day 8	[2, 18]	[2, 18]	NA
Week 4	Day 29	[19, 43]	[19, 43]	NA
Week 8	Day 57	[44, 71]	[44, 71]	NA
Week 12	Day 85	[72, 99]	[72, 99]	NA
Week 16	Day 113	[100, 127]	[100, 127]	NA
Week 20	Day 141	[128, 155]	[128, 155]	NA
Week 24	Day 169	[156, 211]	[156, 211]	≥85

Week 28	Day 197	NA	NA	NA
Week 32	Day 225	NA	NA	NA
Week 36	Day 253	≥212	≥212	NA

Table 7.2.b Analysis Visit Window for Exploratory Biomarkers

Visit	Scheduled Day	Soluble MAdCAM-1, Liver chemistry[a]	Liver biopsy[b]	HepQuant-SHUNT DSI, Child-Pugh score, MELD score	hsCRP, calprotectin	LSM, CAP, Agile 4 score, FAST score, FIB-4 score	Immunogenicity[c]
Baseline (Part 1)	Day -7	NA	NA	NA	NA	NA	NA
Baseline (Part 2)	Day 1	1	NA	1	1	1	1
Week 1	Day 8	[2, 18]	NA	NA	NA	NA	NA
Week 4	Day 29	[19, 43]	NA	NA	[2, 43]	NA	NA
Week 8	Day 57	[44, 71]	NA	NA	[44, 71]	NA	NA
Week 12	Day 85	[72, 99]	NA	NA	[72, 99]	[2, 127]	[2, 127]
Week 16	Day 113	[100, 127]	NA	NA	[100, 127]	NA	NA
Week 20	Day 141	[128, 155]	NA	NA	[128, 155]	NA	NA
Week 24	Day 169	[156, 211]	≥85	≥85	[156, 211]	≥128	[128, 183]
Week 28	Day 197	NA	NA	NA	NA	NA	[184, 211]
Week 32	Day 225	NA	NA	NA	NA	NA	[212, 239]
Week 36	Day 253	≥212	NA	NA	≥212	NA	≥240

[a] Liver chemistry includes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TBL).

[b] Baseline liver biopsy is collected during screening.

[c] Immunogenicity includes antidrug antibodies (ADA).

Table 7.2.c Analysis Visit Window for Safety Assessments

Visit	Scheduled Day	Vital signs, body weight	12-lead ECG	Laboratory tests[a]
Baseline (Part 1)	Day -7	NA	NA	NA
Baseline (Part 2)	Day 1	1	1	1
Week 1	Day 8	NA	NA	[2, 18]
Week 4	Day 29	[2, 43]	NA	[19, 43]
Week 8	Day 57	[44, 71]	NA	[44, 71]
Week 12	Day 85	[72, 99]	NA	[72, 99]
Week 16	Day 113	[100, 127]	NA	[100, 127]
Week 20	Day 141	[128, 155]	NA	[128, 155]
Week 24	Day 169	[156, 183]	≥85	[156, 211]
Week 28	Day 197	[184, 211]	NA	NA
Week 32	Day 225	[212, 239]	NA	NA
Week 36	Day 253	≥240	NA	≥212

[a] Laboratory tests includes clinical chemistry and hematology tests.

8.3 Appendix B - Missing/Partial Dates for Adverse Event/Concomitant Medication

Missing data imputation for start dates:

If the stop date is non-missing and the imputed start date is after the stop date, the stop date will be used as the start date.

(1) Missing day only

- If the month and year are the same as the month and year of the first dose date, the first dose date will be used.
- If the month and year are before the month and year of the first dose date, the last day of the month will be assigned to the missing day.
- If the month and year are after the month and year of the first dose date, the first day of the month will be assigned to the missing day.

(2) Missing day and month

- If the year is the same as the year of the first dose date, the first dose date will be used.
- If the year is prior to the year of the first dose date, December 31st will be assigned to the missing fields.
- If the year is after the year of the first dose date, January 1st will be assigned to the missing fields.

(3) Missing day, month, and year

- The first dose date will be used.

Missing data imputation for stop dates:

If the start date is non-missing and the imputed stop date is before the start date, the start date will be used. If the death date is available and the imputed stop date is after the death date, the death date will be used.

(1) Missing day only

- The last day of the month will be assigned as the missing day.

(2) Missing day and month

- December 31st will be assigned to the missing fields.

(3) Missing day, month, and year

- Th event will be regarded as ongoing.

8.4 Appendix C – Criteria for Clinically Significant Values

Table 7.4.a Hematology - Criteria for Clinically Significant Values

Parameter	Unit	Low Abnormal	High Abnormal
Hemoglobin	Both	<0.8 × LLN	>1.2 × ULN
Hematocrit	Both	<0.8 × LLN	>1.2 × ULN
RBC count	Both	<0.8 × LLN	>1.2 × ULN
WBC count	Both	<0.5 × LLN	>1.5 × ULN
Platelet count	Conventional SI	$<75 \times 10^3/\mu L$ $<75 \times 10^9/L$	$>600 \times 10^3/\mu L$ $>600 \times 10^9/L$

LLN=lower limit of normal, ULN=upper limit of normal, RBC=red blood cell, WBC=white blood cell.

Table 7.4.b Clinical Chemistry - Criteria for Clinically Significant Values

Parameter	Unit	Low Abnormal	High Abnormal
ALT	Both	--	>3 × ULN
AST	Both	--	>3 × ULN
GGT	Both	--	>3 × ULN if baseline is normal; >2 × baseline if baseline is high abnormal
Alkaline phosphatase	Both	--	>3 × ULN if baseline is normal; >2 × baseline if baseline is high abnormal
Calcium	Conventional SI	<7.0 mg/dL <1.75 mmol/L	>11.5 mg/dL >2.88 mmol/L
Chloride	Conventional SI	< 75 mEq/L <75 mmol/L	>126 mEq/L >126 mmol/L
Total bilirubin	Both	--	>1.5 × ULN if baseline is normal, >1.5 × baseline if baseline is high abnormal
Albumin	Conventional SI	<2.5 g/dL <25 g/L	-- --

Total protein	Both	<0.8 × LLN	>1.2 × ULN
Creatinine	Conventional SI	-- --	>2.0 mg/dL >177 µmol/L
Blood urea nitrogen	Conventional SI	-- --	>30 mg/dL >10.7 mmol/L
Sodium	Conventional SI	<130 mEq/L <130 mmol/L	>150 mEq/L >150 mmol/L
Potassium	Conventional SI	<3.0 mEq/L <3.0 mmol/L	>5.5 mEq/L >5.5 mmol/L
Glucose	Conventional SI	<50 mg/dL <2.8 mmol/L	>180 mg/dL >10 mmol/L
Bicarbonate	Conventional SI	<8.0 mEq/L <8.0 mmol/L	-- --
Creatine kinase	Both	--	>ULN – 2.5 × ULN
Total cholesterol	Conventional SI	-- --	>300 mg/dL >7.72 mmol/L
INR	Both	--	>1.5 × ULN

ALT=alanine aminotransferase, AST=aspartate aminotransferase, GGT=γ-glutamyl transferase, LLN=lower limit of normal, ULN=upper limit of normal.

Table 7.4.c Vital Signs and Body Weight - Criteria for Clinically Significant Values

Parameter	Unit	Lower Criteria	Upper Criteria
Respiratory rate	breaths/min	<12	>25
Pulse	bpm	<50	>120
Systolic blood pressure	mmHg	<85	>180
Diastolic blood pressure	mmHg	<50	>110
Body temperature	°C	<35.6	>37.7
Body weight	kg	Change of ≥7% body weight	

9.0 ELECTRONIC SIGNATURE

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