Enterome: EOCRC1-22 (EN05)

Statistical Analysis Plan

Date:	**06-Mar-2024**
Version:	**v01**
Status:	**final**
Author:	[redacted]

Study Title:	A phase 2 trial of EO2040, a miCrobiaL-derived peptide therApeUtic vaccine, in combination with nivolumab, for treatment of patients with circulating tumor DNA-dEfined minimal residual disease of colorectal cancer stage II, III, or IV after completion of curative therapy (the "CLAUDE" study).
Investigational Product:	EO2040
Clinical Phase:	II
Enrolment of first patient	19-Jan-2023 (actual date)
Last Patient Last Visit	23-Jan-2024 (actual date)
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Signatures

[redacted]

Table of Contents

1 DOCUMENT HISTORY

Version	Date	Author / editor of new version	Main changes / comments
v01	06-Mar-2024	[redacted]	First version

2 LIST OF ABBREVIATIONS

Abbreviation	Text
AE	Adverse Events
APS	All-Patient Set
ATC	Anatomical Therapeutic Chemical
BIRC5	Baculoviral Inhibitor of Apoptosis Repeat-containing5
CRC	Colorectal cancer
CRF	Case Report Form
CSR	Clinical Study Report
ctDNA	Circulating tumor deoxyribonucleic acid
DFS	Disease-free survival
DNA	Deoxyribonucleic acid
eCRF	Electronic Case Report Form
ELISpot	Enzyme-linked Immunospot
FAS	Full Analysis Set
FOXM1	forkheadboxM1
ICH	International Council for Harmonization
IFN-γ	Interferon-gamma
IHC	Immunohistochemistry
MDSC	Myeloid-derived suppressor cells
MedDRA	Medical Dictionary for Regulatory Activities
MRD	Minimal residual disease
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for AE
OS	Overall Survival
PD-L1	Programmed Death-Ligand 1
PPS	Per-Protocol Set
PT	Preferred Term
QC	Quality control
SAE	Serious Adverse Event
SOC	Standard of Care, System Organ Class
SOP	Standard Operating Procedure
SS	Safety Set
TAA	Tumor associated antigens
TCR	T cell Receptor
TEAE	Treatment-Emergent Adverse Events
UCP2	Universal Cancer Peptide 2
WHODD	World Health Organization Drug Dictionary

3 GENERAL

This statistical analysis plan reflects study protocol EOCRC1-22 dated 04-JUL-2022. It follows the principles of the Guidelines International Council for Harmonization (ICH) Topic E3 and ICH Topic E9. It gives all details for the final statistical analysis of this study.

3.1 Analyses Planned and Already Performed

No formal analysis of the data will be performed as the study is planned to be terminated with just one treated patient, only patient data listings will be produced.

3.2 SOPs to be Followed

The analysis will be carried out according to Metronomia Standard Operation Procedures (SOPs).

The report will be written according to the ICH E3 and other applicable ICH guidelines.

4 OVERVIEW OF THE PROTOCOL

4.1 Study Objectives

4.1.1 Primary Objective

- The primary objective of this trial is to assess the 6-month circulating tumor deoxyribonucleic acid (ctDNA) clearance rate at therapy with EO2040 in combination with nivolumab, in patients with ctDNA-defined minimal residual disease (MRD) of stage II-IV colorectal cancer after completion of curative therapy.

and in sequence, provided the first objective has a positive outcome per protocol Section 9:

- The primary objective is, to assess the 6-month ctDNA clearance rate at therapy with EO2040 monotherapy, in patients with ctDNA-defined MRD of stage II-IV colorectal cancer after completion of curative therapy.
    - *In case the first objective does not have a positive outcome per protocol Section 9, a protocol amendment to adjust the second part of the primary objective to utilize an alternative therapeutic vaccine than EO2040 will be considered.*

ctDNA clearance is utilized as a surrogate endpoint for eventual cure, and thereby prolongation of disease-free survival (DFS) for patients achieving clearance.

Note, clearance of ctDNA is characterized by the disappearance of all somatic mutations identified in the blood, as well as no appearance of any additional new somatic mutations, and radiographic investigation(s) showing no evidence of colorectal cancer (see protocol Section 7.8).

4.1.2 Secondary Objectives

To assess the following items at therapy with EO2040 in combination with nivolumab, and when applicable EO2040 monotherapy, in patients with ctDNA-defined MRD of stage II-IV colorectal cancer after completion of curative therapy:

- Safety and tolerability of study treatments,
- The 3-month ctDNA clearance rate,

- Progression of colorectal cancer and death as DFS,
- Overall Survival (OS),
- Survival at 36 months after start of study therapy, and
- Induction/expansion of T cells specific for EO2040, the components of EO2040, and the targeted nominal tumor associated antigens (TAAs) (Baculoviral Inhibitor of Apoptosis Repeat-containing5 [BIRC5] and forkheadboxM1 [FOXM1]).

4.1.3 Exploratory Objectives

[redacted]

- To assess correlations between immunogenicity of EO2040, the components of EO2040, and the targeted nominal TAAs (BIRC5 and FOXM1) and efficacy, and safety, outcome parameters.

[redacted]

4.2 Study Endpoints

4.2.1 Primary Endpoint

- The primary endpoint is response to treatment at 6 months (see protocol Section 3.1.1), which is defined as having clearance of ctDNA, AND not having any radiographic evidence of recurrence at 6 months (see also protocol Section 7.8 for rules of assessment of an individual patient best overall response).

Clearance of ctDNA will be characterized by the disappearance of all somatic mutations

identified in the blood, as well as no appearance of any additional new somatic mutations.

4.2.2 Secondary Endpoint

- The safety and tolerability of study treatments will be determined by a descriptive medical assessment of the combined profile of incidences of adverse events (AEs), treatment-emergent AEs (TEAEs), serious AEs (SAEs), deaths, reasons for treatment discontinuation/delays, and laboratory abnormalities using the NCI-CTCAE v5.0 grading system (see Section 11.3 below and Section 7.7 in protocol).
- Response to therapy at 3 months, defined as clearance of ctDNA, AND no radiographic evidence of recurrence (determination method will be the same as for the primary endpoint; see protocol Section 3.2.1).
- DFS defined as the time from start of study treatment to the date of first documented CRC recurrence or death due to any cause, whichever occurs first. Patients alive without recurrence will be censored at the date of last follow-up time. Recurrence is defined by the appearance of one or more new CRC lesions on imaging and/or other clinical evaluation such as endoscopy.
- OS, measured as the time from start of study treatment until death from any cause. Patients alive will be censored at the last time documented to be alive.
- Survival at 36 months after start of study treatment, estimated via Kaplan-Meier estimates.
- Immunogenicity and cross reactivity: immunogenicity determined as expansion of specific T cells comparing samples taken at baseline versus on treatment in an individual patient [redacted]

4.2.3 Exploratory Endpoint

[redacted]

[redacted]

- Correlations between immunogenicity [redacted] of EO2317, EO2318, and UCP2 and clinical efficacy (per primary and secondary efficacy endpoints) and safety (TEAEs of defined specificities and grades) outcome parameters. Cross reactivities shown for the TAAs BIRC5/survivin, and FOXM1 will also be explored in the same way.

[redacted]

4.3 Study Design

The trial is a multi-center, open-label, non-comparative, two sequential cohort, phase 2 trial, to investigate as the first cohort efficacy of the microbiome-derived therapeutic vaccine EO2040 in combination with nivolumab in patients with stage II-IV colorectal cancer with ctDNA defined MRD after completion of curative therapy. Assuming a positive outcome of the first cohort, a second cohort assessing efficacy of EO2040 monotherapy, with the option of addition of nivolumab after 3 months in case of no ctDNA clearance, is planned.

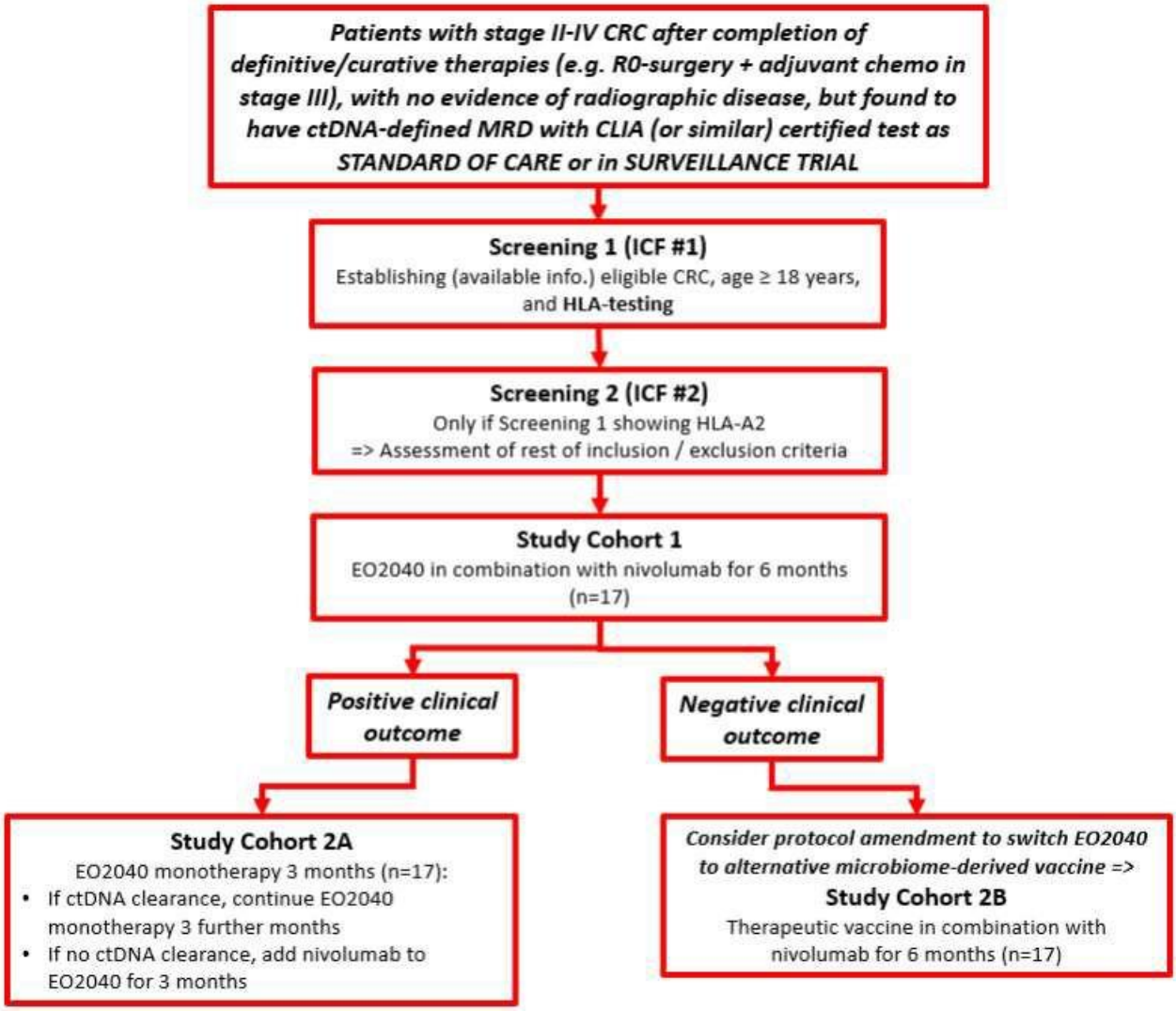
The study is assumed to recruit a total of 17 patients in Cohort 1, and if cohort 1 has a positive outcome (see protocol Section 9), a total of 17 patients will also be recruited to Cohort 2A.

[redacted]

[redacted]

Schematic overview of the trial design, and an overview of the treatment schedule are illustrated in figures below.

Trial design overview:



Treatment schedule, scan and ctDNA assessments, and blood sampling for immune testing and serum collection:

[redacted]

4.4 Sample Size

Considering prior attempts in similar situations, the trial will include 17 patients per cohort. The primary objective of the trial is to determine the ctDNA clearance rate at 6 months (R6).

[redacted]

4.5 Study Flow Chart

Refer the latest study protocol.

5 GENERAL ASPECTS OF THE STATISTICAL ANALYSIS

5.1 Analysis Sets

The **All-Patient (AP) set** will consist of any patient who signed informed consent including screen-failures.

The **Full Analysis Set (FAS)** will consist of patients who received at least one dose of EO2040 and for whom no important protocol deviations occurred that would compromise the evaluation of efficacy.

The **Safety Set (SS)** will consist of patients who received at least one dose of study treatment.

[redacted]

5.2 Protocol Devations

All protocol deviations regardless of their source will be listed for each patient together with the categorization into major or minor.

5.3 Intercurrent Events

Not Applicable.

5.4 Changes or Deviations from Planned Analyses

Since the study is planned to be terminated with just one treated patient, no formal analysis of the data will be performed.

6 DEFINITIONS FOR STATISTICAL ANALYSIS

6.1 Baseline and Change from Baseline

Baseline is defined as the last non-missing value prior to first administration of study treatment.

Absolute Change from Baseline at a visit/timepoint will be calculated as follows:

Result at visit/timepoint – Baseline result;

For assessments taken before and after first administration of study treatment, baseline assessments will be flagged separately in the listings. Similarly, change from baseline values will be displayed alongside observed results in the respective listings.

6.2 Study Day

The reference day (Day 1) for study day calculations is the date of the first administration (V1) of study treatment. The study day will be calculated as follows:

- Assessment/Visit Date – Date of first administration study treatment + 1; for assessments on or after the date of first administration of study treatment
- Assessment/Visit Date – Date of first administration study treatment; for assessments before the date of first administration of study treatment

There is no Study Day 0.

Study day will be displayed alongside visit/event dates in the relevant listings.

6.3 Duration

Duration of an event will be calculated as follows:

Duration (days) = (End date of event – Start date of event) +1;

In case of partial start/end dates, duration will be calculated assuming the 15th of the respective months if case of missing day. Duration will not be calculated if months and day are not given.

All listings displaying event data like AE, medication, etc., will include duration of the events alongside the end dates.

6.4 Visit windows and Analysis Visits

Data collected by visit will be displayed for the visit it was collected, irrespective of the actual date of the assessment.

The following tables shows the visit labels to be used in patient data listings:

7 STATISTICAL ANALYSIS SPECIFICATION

7.1 Specifications Related to Whole Analysis

7.1.1 Tables

No tables will be produced.

7.1.2 Data Listings

All CRF data will be listed as documented. For screen failures only demographic data and screen failure details will be listed. Relevant generated variables from section 6 will be listed

next to the original data items as well.

In all listings, the cohort labels will be displayed as given below:

- Cohort 1
- Cohort 2A
- Cohort 2B

In listings with screen failure data, screen failures will have cohort label as “Not Applicable” and will be sorted at last.

The listings will be sorted by cohort, patient identifier, visit and date (if applicable).

Medical history, Procedures and Adverse event:

All medical history, concomitant illness, procedures and adverse events will be coded using MedDRA version mentioned in the latest data management plan and system organ class (SOC), preferred term (PT) of the events will be presented in the listing.

Medication:

All medication will be coded using World Health Organization Drug Dictionary (WHODD) version mentioned in the latest data management plan and Anatomical Therapeutic Chemical (ATC) level 2 term, PT of the medications will be presented in the listing.

8 SOFTWARE AND STATISTICAL PROGRAMMING

The statistical analysis will be performed using the SAS® statistical software package in Version 9.4.

SAS programming will be performed according to Metronomia standards as defined in BM-08-SOP “Statistical Analysis and Programming” and related work instructions. Special attention will be paid to planning and performance of quality control measures as documented in the QC plan for the analysis of this study (see also BM-08-WIN03 “How to Plan and Document QC for Statistical Analysis”).

9 REFERENCES

Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, Published: November 27, 2017. U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, National Institutes of Health National Cancer Institute, https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference_5x7.pdf. Accessed February 2, 2024.

ICH Topic E9: Statistical Principles for Clinical Trials, 5 February 1998, adopted by CPMP, March 1998, issued as CPMP/ICH/363/96ICH

ICH E9 (R1): Addendum on Estimands and Sensitivity Analysis in Clinical Trials to the Guideline on Statistical Principles for Clinical Trials, 20 November 2019, adopted by CHMP, 30 January 2020, issued as EMA/CHMP/ICH/436221/2017

SAS 9.4 https://support.sas.com/documentation/94/index.html. Copyright © SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA. All rights reserved.

10 CHANGES TO FINAL VERSION OF THE SAP

Not Applicable.

11 LIST OF TABLES, FIGURES AND LISTINGS

11.1 Table of Contents for End-of-Text Tables and Figures

No tables and figures will be produced.

11.2 Table of Contents of Individual Patient Data Listings (Appendix 16.2 of the CSR)

[redacted]

[redacted]

11.3 Grading of safety laboratory parameters analogous to NCI CTCAE v5.0

[redacted]

[redacted]