Clinical Study Report Synopsis

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 "CL AUDE" study)*

Investigational Product	EO2040
EudraCT number	2022-002679-12
US IND	28389
Indication	Treatment of patients with ctDNA-defined minimal residual desease of colorectal cancer stage II, III or IV
Phase	II
Study initiation date	2023-Jan-19
Study early termination date	2024-Jan-23
Date of report	2025-Jan-02

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This study was performed in compliance with Good Clinical Practices, including the archiving of essential documents. This report has been prepared in accordance with the ICH Harmonized Tripartite Guideline on the Structure and Content of Clinical Study Reports, dated July 1996.

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Title of Study: 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*).

Name of Product: EO2040

Principal Investigator: Dr Arvind Dasari, MD, MS, Associate Professor

Study center: Department of Gastroenterology Medical Oncology, Division of Cancer Medicine, The University of Texas, MD Anderson Cancer Center, Houston, TX, USA

Publication: No data published

Phase of development: II

Studied period: Date of first enrolment (first signing of ICF): 2023-Jan-19; date of last completed (last patient last visit): 2024-Jan-23

Objectives: The primary objective of this trial was to assess the 6-month 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 (CRC) after completion of curative therapy.

The secondary objectives were to assess safety and tolerability of study treatment, the 3-month ctDNA clearance rate, progression of colorectal cancer and death as disease-free survival (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 TAAs (BIRC5 and FOXM1).

Methodology: This was a multi-center, open-label, non-comparative two sequential cohorts, phase II 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 (see Primary Endpoint), a second cohort assessed efficacy of EO2040 monotherapy, with the option of addition of nivolumab after 3 months in case of no ctDNA clearance was planned.

Number of patients (planned and analyzed): Of the 34 patients who were planned to be treated (17 patients in each of two sequential cohorts), only one patient in the US was treated and included in the analysis.

Diagnosis and main criteria for inclusion: HLA-A2 positive patients with stage II-IV colorectal cancer with ctDNA defined MRD after completion of curative therapy, at an age ≥ 18 years, with ECOG performance status 0 to 1.

Test product, dose and mode of administration, duration of treatment: EO2040, is a therapeutic peptide vaccine composed of two microbial-derived peptides mimicking cytotoxic T cell (CD8+ T cell) epitopes from the TAAs BIRC5/survivin and FOXM1, combined with the helper peptide (CD4+ T cell epitope) Universal Cancer Peptide 2, UCP2.

The peptide mix EO2040 (final concentration of each peptide is 300 µg/mL), i.e. drug product (DP) is emulsified with the adjuvant Montanide™ ISA 51 VG to form a water-in-oil emulsion before subcutaneous (SC) administration.

EO2040 was given in combination with nivolumab, which is an anti-PD1 fully human monoclonal antibody (immunoglobulin G4), blocking the interaction between PD1 and its ligands (PD-L1 and PD-L2), in another group of patients EO2040 was also planned to be evaluated as monotherapy.

Nivolumab is approved for use for the treatment of multiple cancer types, including subtypes of CRC (mismatch repair deficient or microsatellite instability-high metastatic disease after prior treatment). However, it is not currently approved for ctDNA defined MRD of CRC. Treatment with nivolumab followed the current versions of European SmPC and US PI.

The selected treatment duration was 6 months, based on mechanistical considerations regarding the study treatment, and the ease of timing with standard of care procedures as further ctDNA testing and radiographic imaging.

Statistical methods planned: No specific statistical method was applied. A descriptive analysis has been performed since only one patient was treated in this trial. All data collected in the trial are reported in Patient Data Listing by summary tables for all demographic and baseline characteristics, medical history, efficacy, and safety variables.

Conclusion: The study was planned to recruit approximately 34 evaluable patients, but due to lack of recruitment only one patient was enrolled and treated leading to early study termination.

In total, during the study, the patient has received 3 injections (at visits 1, 2 and 5) of EO4010 with Montanide ISA 51 VG in combination with nivolumab.

The patient experienced 7 treatment emergent adverse events (4 G1, 2 G2; 1 G3), all events recovered but one, hypothyroidism, treated with levothyroxine. Among 7 events, 1 event (local administration site reaction G3) was considered serious and related to EO4010 leading to study treatment interruption, and 1 event (pyrexia G1) was considered related to nivolumab. In addition, the patient developed hyperthyroidism (thyroid stimulating hormone decreased G1) converting to hypothyroidism G2 treated with levothyroxine, as well as papulopustular rash G2 recovered after use of steroids. These 3 events were assessed as not related to study treatment (EO4010 or nivolumab) by the investigator, but a potential immune mediated mechanism related to nivolumab cannot be fully ruled out.

Based on the data above, the patient's safety profile seems to be consistent with the safety profile of nivolumab monotherapy as per EMA and FDA labelling, except the addition of local administration site reactions. Administration site reaction, including severe events, has been described for several antigens administered together with Montanide ISA 51 VG as an adjuvant (van Doorn et al). No new safety signal has been raised from the data collected in the study.

The immunogenicity data demonstrated good reactivity against some bacterial and human peptides: $CD8^+$ specific T cells for EO2317, EO2318 and BIRC5 have been detected *ex vivo* after only 1 administration of EO2040 (V2; week 3) with highest frequency for EO2317 (0.74% of $CD8^+$ T cells), EO2318 (0.74% of $CD8^+$ T cells), and BIRC5 (0.5% of $CD8^+$ T cells) at V4 (week 7). Functional $CD8^+$ T cells are detected at visit 6(week 13) in response to bacterial (134 spots/10^6 cells), human (53 spots/10^6 cells), and UCP2 (89 spots/10^6 cells) peptides.

CD8$^+$ specific T cells for EO2317, EO2318, BIRC5 and FOXM1 have been detected after IVS after 1 administration of EO2040 (visit 2; week 3), with highest frequency for EO2317 (1.32% of CD8$^+$ T cells) and BIRC5 (0.36% of CD8$^+$ T cells) at visit 2 (week 3) and 2318 (12.07% of CD8$^+$ T cells) and FOXM1 (0.28% of CD8$^+$ T cells) at visit 5 (week 11).

Functional CD8$^+$ T cells have been detected in response to bacterial (3113 spots/10^6 cells at visit 5; (week 11) and human (1073 spots/10^6 cells at V5; week 11) peptide pool and UCP2 peptide (4700 spots/10^6 cells at visit 5 (week 11).

While the level of immune responses observed already after one administration at visit 2 (week 3) and 2 administrations at visit 4 (week 7), no administration was done at visit 3 (week 5). Administrations of EO2040, could explain the grade 3 local administration site reaction, pausing of treatment at visit 3 (week 5) and visit 4 (week 7) might have reduce the possibility to activate enough CD8 cells to exert cytotoxic activity against tumoral cells. However, based on the limited data collected in one patient with rapid radiological disease progression occurring 3.2 months after treatment initiation, no sustained conclusion regarding efficacy results can be given.

Conduct of clinical studies using a noninvasive approach such as ctDNA outcome measures to explore new therapeutic options and to potentially show eradication of micrometastatic disease in high-risk patients seems to be relevant, even though the current study showed problems with recruitment despite significant efforts during around 10 months in the USA. Recruitment in the USA was pre- considered to be easier than in Europe since ctDNA testing is becoming a standard of care (in this study population) and also testing outside studies was reimbursed by insurance companies. It is assumed, that the study recruitment was significantly behind expectations due to rarity of patients with ctDNA positivity per the protocol inclusion criteria, rapid radiological disease progression in patients who start screening, and the percentage of HLA-A2 negative patients being higher than expected. These issues should be taken into consideration for further development, if any, of EO2040 in the ctDNA setting.