# Clinical Trial Protocol EOCRC1-22

## 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 |
| EU trial number: | 2022-500664-35-00 |
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Property of the Sponsor:
Enterome
94/96 avenue Ledru-Rollin 75011
Paris, France

Sponsor Signatory & Medical Monitor:
[redacted]
Chief Medical Officer, Enterome

**Enterome Medical Monitor Telephone** [redacted]
**Enterome Medical Monitor E-Mail** [redacted]

# SIGNATURE FOR PROTOCOL APPROVAL AND RELEASE

I, the undersigned, have read this protocol and the appendices and agree that they contain all the necessary information required for the conduct of the study.

[redacted]
Chief Medical Officer
Enterome, Paris, France

Date

**Sponsor information**

(Achille) **CLAUDE** Debussy (22 August 1862 – 25 March 1918) was a French composer. He is sometimes seen as the first impressionist composer, although he vigorously rejected the term. He was among the most influential composers of the late 19th and early 20th centuries.

Born to a family of modest means and little cultural involvement, Debussy showed enough musical talent to be admitted at the age of ten to France's leading music college, the Conservatoire de Paris. He originally studied the piano, but found his vocation in innovative composition, despite the disapproval of the Conservatoire's conservative professors. He took many years to develop his mature style and was nearly 40 when he achieved international fame in 1902 with the only opera he completed, *Pelléas et Mélisande*.

Debussy's orchestral works include *Prélude à l'après-midi d'un faune* (1894), *Nocturnes* (1897–1899) and *Images* (1905–1912). His music was to a considerable extent a reaction against Wagner and the German musical tradition. He regarded the classical symphony as obsolete and sought an alternative in his "symphonic sketches", *La mer* (1903–1905). His piano works include sets of 24 Préludes and 12 Études. Throughout his career he wrote mélodies based on a wide variety of poetry, including his own. He was greatly influenced by the Symbolist poetic movement of the later 19th century. A small number of works, including the early *La Damoiselle élue* and the late *Le Martyre de saint Sébastien* have important parts for chorus. In his final years, he focused on chamber music, completing three of six planned sonatas for different combinations of instruments.

With early influences including Russian and Far Eastern music, Debussy developed his own style of harmony and orchestral coloring, derided – and unsuccessfully resisted – by much of the musical establishment of the day. His works have strongly influenced a wide range of composers including Béla Bartók, Olivier Messiaen, George Benjamin, and the jazz pianist and composer Bill Evans. Debussy died from colorectal cancer at his home in Paris at the age of 55 after a composing career of a little more than 30 years.

# GLOBAL COORDINATING INVESTIGATOR AGREEMENT

I, the undersigned, have read this protocol and the appendices and agree that they contain all the necessary information required for the conduct of the study, and I agree to conduct the study as described herein.

[redacted] Date

Associate Professor, Department of
Gastrointestinal (GI) Medical Oncology,
Division of Cancer Medicine,
The University of Texas MD Anderson Cancer Center,
Houston, TX, USA

# EUROPEAN COORDINATING INVESTIGATOR AGREEMENT

I, the undersigned, have read this protocol and the appendices and agree that they contain all the necessary information required for the conduct of the study, and I agree to conduct the study as described herein.

_____ _____

[redacted]
Department of Oncology,
University Hospital Carl Gustav Carus,
Dresden, Germany

Date

# SITE PRINCIPAL INVESTIGATOR AGREEMENT

I, the undersigned, have read this protocol and the appendices and agree to conduct the study as described herein.

Printed Name: _____

Site location: _____

Signature: _____

Date: _____

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## ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| ACC | Adrenocortical Carcinoma |
| ACTH | Adrenocorticotropic hormone |
| AE | Adverse Events |
| AESI | Adverse Events of Special Interest |
| AIDS | Acquired Immunodeficiency Syndrome |
| ALP | Alkaline Phosphatase |
| ALT | Alanine Aminotransferase |
| ANC | Absolute Neutrophil Counts |
| Anti-HBcAg | Antibody to the Hepatitis B Core Antigen |
| Anti-HBsAg | Antibody to the Hepatitis B Surface Antigen |
| AP-1 | Activator Protein 1 |
| APC | Antigen Presenting Cell |
| APTT | Activated Partial Thromboplastin Time |
| ASCO | American Society of Clinical Oncology |
| AST | Aspartate Aminotransferase |
| BIRC5 | Baculoviral Inhibitor of Apoptosis Repeat-containing5 |
| BOR | Best Overall Response |
| BP | Blood Pressure |
| bpm | Beats per Minute |
| BSA | Body Surface Area |
| BUN | Blood Urea Nitrogen |
| CAPOX | Capecitabine-oxaliplatin |
| CEA | Carcinoembryonic antigen |
| CFR | Code of Federal Regulations |
| CI | Confidence Interval |
| CK | Creatine Kinase |
| CLIA | Clinical Laboratory Improvement Amendments |
| CNS | Central Nervous System |
| COAD | Colon adenocarcinoma |
| COVID | Corona Virus Disease |
| CR | Complete Response |
| CRAs | Clinical Research Associates |
| CRC | Colorectal cancer |
| CRO | Clinical Research Organisation |
| CRP | C-reactive Protein |
| CSR | Clinical Study Report |
| CT | Computed Tomography |
| ctDNA | Circulating tumor deoxyribonucleic acid |
| CTLA-4 | Cytotoxic T-lymphocyte-associated Antigen 4 |
| DC | Dendritic Cells |
| DFS | Disease-free survival |
| DL | Dose Level |

| | |
|---|---|
| [redacted] | [redacted] |
| DP | Drug Product |
| EBV | Epstein Barr Virus |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | Electronic Case Report Form |
| EDC | Electronic Data Capture |
| ELISA | Enzyme-linked Immunosorbent Assay |
| ELISpot | Enzyme-linked Immunospot |
| ENT | Ears, Nose, Throat |
| EORTC | European Organisation for Research and Treatment of Cancer |
| ESMO | European Society for Medical Oncology |
| FACS | Fluorescence-activated Cell Sorting |
| FAS | Full Analysis Set |
| FD&C | Food Drug & Cosmetic |
| FDG | FluoroDeoxyGlucose |
| FOLFOX | Leucovorin/5-fluorouracil/oxaliplatin |
| FOXM1 | forkheadboxM1 |
| FPI | First Patient Included |
| FSH | Follicle-stimulating Hormone |
| FVC | Forced Vital Capacity |
| GB | Glioblastoma |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| GTEX | Genotype-Tissue Expression |
| Hb | Hemoglobin |
| HBsAg | Hepatitis B Surface Antigen |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C virus |
| HIV | Human Immunodeficiency Virus |
| HLA | Human Leukocyte Antigen |
| HR | Heart Rate |
| hTERT | human Telomerase Reverse Transcriptase |
| IB | Investigator's Brochure |
| ICF | Informed Consent Form |
| ICH | International Council for Harmonization |
| ICI | Immune Checkpoint Inhibitor |
| IDMC | Independent Data Monitoring Committee |
| IEC | Independent Ethics Committee |
| IFN | Interferon |
| IFN-γ | Interferon-gamma |
| IHC | Immunohistochemistry |
| IL13Rα2 | Interleukin-13 Receptor Alpha-2 |
| IMP | Investigational Medicinal Product |

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|---|---|
| INR | International Normalized Ratio |
| IR | Incidence Rates |
| IRB | Institutional Review Board |
| ISV | In Situ Vaccine |
| IU | International Unit |
| IUD | Intra Uterine Device |
| IUS | Intra Uterine hormone-releasing System |
| IWRS | Interactive Web Response System |
| LDH | Lactate Dehydrogenase |
| LLN | Lower Limit of Normal |
| LPI | Last Patient Included |
| MDSCs | Myeloid-derived Suppressor Cells |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MESF | Molecules of Equivalent Soluble Fluorochrome |
| MHC | Major histocompatibility complex |
| MMR | Mismatch repair |
| MPP | Malignant Pheochromocytoma/Paraganglioma |
| mRNA | message Ribonucleic Acid |
| MRD | Minimal residual disease |
| MRI | Magnetic resonance imaging |
| MSI | Microsatellite instability |
| NCCN | National Comprehensive Cancer Network |
| NCI-CTCAE | National Cancer Institute-Common Terminology Criteria for AEs |
| NED | No evidence of disease |
| NGS | Next-generation sequencing |
| NSCLC | Non-Small-Cell Lung Cancer |
| NK | Natural Killer |
| OS | Overall Survival |
| [redacted] | [redacted] |
| PCV | Procarbazine, lomustine and vincristine |
| PD | Progressive Disease |
| PD1 | Programmed cell Death protein 1 |
| PDL-1 | Programmed Death-Ligand 1 |
| PET | Positron Emission Tomography, |
| PFS | Progression-free Survival |
| Ph.Eur. | European Pharmacopoeia |
| PI | Principal Investigator |
| PP | Per-Protocol |
| PS | Performance Status |
| PT | Preferred Term |
| PTT | Partial Thromboplastin Time |
| RANO | Response Assessment in Neuro-Oncology |
| Rb | Retinoblastoma |
| RBC | Red Blood Cells |

| | |
|---|---|
| READ | Rectum adenocarcinoma |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SARS | Severe Acute Respiratory Syndrome |
| SC | Subcutaneous |
| SEER | Surveillance, Epidemiology and End Results |
| SEM | Standard error of the mean |
| SmPC | Summary of Product Characteristics |
| SOC | Standard of Care |
| SOC | System Organ Class |
| SOCS | Suppressor of Cytokine Signaling |
| SP | Safety Population |
| SUSARs | Suspected Unexpected Serious Adverse Reactions |
| TAA | Tumor Associated Antigen |
| TCR | T cell Receptor |
| TEAE | Treatment-Emergent Adverse Events |
| TERT | Telomerase Reverse Transcriptase |
| TNF | Tumor Necrosis Factor |
| TNM | Tumor Nodes Metastases |
| TMB | Tumor Mutational Burden |
| TRAE | Treatment-Related Adverse Event |
| TSH | Thyroid-Stimulating Hormone |
| UCP2 | Universal Cancer Peptide 2 |
| ULN | Upper Limit of Normal |
| USP-NF | United States Pharmacopoeia – National Formulary |
| WBCs | White Blood Cells |
| WT | Wild-type |

## SYNOPSIS

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|---|---|
| **Investigational medicinal product** | EO2040, is a therapeutic peptide vaccine composed of two microbial-derived peptides (EO2317 and EO2318) mimicking cytotoxic T cell (CD8+ T cell) HLA-A2 restricted epitopes from two different tumor associated antigens (TAAs), survivin also called baculoviral inhibitor of apoptosis repeat-containing protein 5 (BIRC5), and forkhead box M1 (FOXM1), respectively. EO2040 also includes the helper peptide (CD4+ T cell epitope) universal cancer peptide 2 (UCP2), derived from the human telomerase reverse transcriptase catalytic subunit (hTERT).<br>The peptide mix EO2040, i.e. drug product (DP), [redacted] subcutaneous (SC) administration. EO2040 will also be given in combination with the anti-PD1 monoclonal antibody nivolumab delivered according to global labels.<br>EO2040, the compound to be used in the current trial is a derivative of the compound EO2401. EO2040 includes the same components as EO2401, except the microbiome derived peptide EO2316, mimicking an epitope on the TAA interleukin 13 receptor alpha-2 (IL-13Rα2). The reason to exclude peptide EO2316 from compound EO2040 is that IL-13Rα2 currently does not have a rationale to be used when colorectal cancer is targeted, i.e. expression levels as known today are not high enough.<br>Note, the parent compound of EO2040, i.e. EO2401, in combination with nivolumab, has been investigated in two phase 1/2 trials in more than 100 patients with glioblastoma and adrenal tumors, showing the ability to generate strong systemic immune responses against the targeted TAAs, and being well tolerated; the safety profile being consistent with the profile of the combination partner nivolumab, with the only addition of local administration site reactions (most commonly grade 1, erythema, induration, and sometimes pain). |
| **Protocol number** | EOCRC1-22 |
| **Title** | A phase 2 trial of EO2040, a mi**C**robia**L**-derived peptide ther**A**pe**U**tic vaccine, in combination with nivolumab, for treatment of patients with circulating tumor **D**NA-d**E**fined minimal residual disease of colorectal cancer stage II, III, or IV after completion of curative therapy (*the "CLAUDE" study*). |
| **Background and rationale** | Colorectal cancer (CRC) is the third most common tumor in men and the second in women, accounting for 10% of all tumor types worldwide. Incidence is 25% higher in males and differs greatly between countries. With more than 600,000 deaths estimated each year, CRC is the fourth most common cancer-related cause of death globally.<br>In 2020, an estimated 104,610 new cases of colon cancer and 43,340 cases of rectal cancer was to occur in the USA. During the same year, an estimated 53,200 people will die of colon and rectal cancer combined. The mortality rate in the European Union is 15-20 out of 100,000 in males and 9-14 out of 100,000 in females and has decreased over time, particularly in females. In affected European individuals, 5-year survival ranges from 28.5% to 57% in men and from 30.9% to 60% in women, with a pooled estimation in 23 countries of 46.8% in men and 48.4% in women.<br>After appropriate diagnostic measures surgical intervention is the first key step in treatment of colon cancer. Assuming surgery for cure, i.e. R0-resection with microscopically margin-negative resection and no gross or microscopic tumor remaining in the primary tumor bed, the assessment of risk of recurrence is important in deciding when to recommend systemic adjuvant treatment with the aim of reducing risk of relapse and death. The risk of relapse after colon cancer resection is estimated by integrating the clinicopathological features of the tumor with the molecular marker mismatch repair (MMR)/microsatellite instability (MSI) status.<br>TNM staging remains the most relevant histological criteria for risk assessment after surgery of colon cancer. Reported 5-year survival rates after surgical resection alone are 99% for stage I, 68%-83% for stage II and 45%-65% for stage III disease. |

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| | In general, it has been established that adjuvant systemic therapy decreases the risk of death by an absolute 3%-5% in high-risk stage II colon cancer with single-agent 5-fluorouracil (5-FU) and by 10%-15% in stage III disease with fluoropyrimidines alone, with a further 4%-5% improvement with oxaliplatin-containing combinations.<br><br>FOLFOX (leucovorin/5-fluorouracil/oxaliplatin) and CAPOX (capecitabine plus oxaliplatin) remain the current standard of care adjuvant treatments for stage III disease. Note, irinotecan, cetuximab and bevacizumab have not demonstrated clinical activity in the localized disease setting and therefore they should never be used as adjuvant treatment in this setting.<br><br>Studies of selected patients undergoing surgery to remove CRC liver metastases (i.e. stage IV) have shown that cure is possible in this population and should be the goal for a substantial number of these patients. Reports have shown 5-year disease-free survival (DFS) rates of approximately 20% in patients who have undergone resection of liver metastases, and a recent meta-analysis reported a median 5-year survival of 38%.<br><br>Despite all efforts regarding surgery and adjuvant therapy per above, approximately 25% of patients who present with localized disease will later develop metastases. In addition, of new colorectal cancer diagnoses, 20% of patients have metastatic disease already at presentation.<br><br>Metastatic disease not amenable to local treatment only (e.g. surgery, stereotactic body radiation therapy, haptic arterial infusion, arterial embolization therapy, tumor ablation via for instance radiofrequency ablation or cryotherapy and other means), is usually treated with systemic chemotherapy or targeted treatments as outlined in different guidance documents.<br><br>In addition to fluoropyrimidine-, oxaliplatin-, and/or irinotecan-containing chemotherapy regimens, immunotherapy and targeted therapy regimens are becoming an increasingly important part of the treatment landscape for patients with metastatic CRC. Combination of a biologic agent (e.g. bevacizumab, cetuximab, panitumumab) with some of the chemotherapy regimens is an option, depending on available data. Systemic therapy options for patients with progressive disease depend on the choice of initial therapy and biomarker status of the tumor.<br><br>As outlined in the ESMO guidance regarding metastatic CRC, a patient with classical metastatic CRC may typically achieve an OS of approximately 30 months as the result of a multidisciplinary team-managed 'continuum of care'. Among people diagnosed with metastatic colorectal cancer, approximately 70%-75% of patients survive beyond 1 year, 30%-35% beyond 3 years, and fewer than 20% beyond 5 years from diagnosis.<br><br>***Thus, CRC continues to be a major therapeutic challenge with a considerable number of patients experiencing premature death from early disease recurrence. Historically, it has been challenging to definitively identify patients with CRC at risk of recurrence after completed assumed curative treatment, which has complicated the ability to optimize adjuvant drug development. Relapsed CRC may occur in the absence of symptoms, further emphasizing the importance of sensitive screening modalities.***<br><br>Circulating tumor DNA (ctDNA) can be detected at varying concentrations across multiple cancer types and disease stages. ctDNA typically constitutes a small proportion of an individual's total circulating cell-free DNA (cfDNA), <1% according to some studies. However, with improving assay techniques providing greater levels of sensitivity, the analysis of ctDNA is rapidly being accepted as a reliable tool in oncology.<br><br>For patients with early-stage solid tumors, ctDNA testing may identify patients who have residual disease or recurrence after potentially curative treatments (surgery in particular). Based on this evidence, multiple clinical trials have been initiated to evaluate the clinical validity and utility of this approach.<br><br>An analysis from the large GALAXY study, an observational study monitoring minimal residual disease, MRD, evaluating the association of ctDNA dynamics with a short-term clinical outcome and adjuvant chemotherapy efficacy was recently presented. A total 1,365 patients with CRC were enrolled between June 2020 and April 2021 and included in the analysis; 116 stage I, 478 stage II, 503 stage III, and 268 oligometastatic resectable |

stage IV. The analysis demonstrated the association of ctDNA dynamics with improved clinical outcomes in MRD+ patients; also showing that stratifying post-surgical treatment decisions using a ctDNA assay can identify patients likely to benefit from adjuvant chemotherapy across all stages, including stage II.

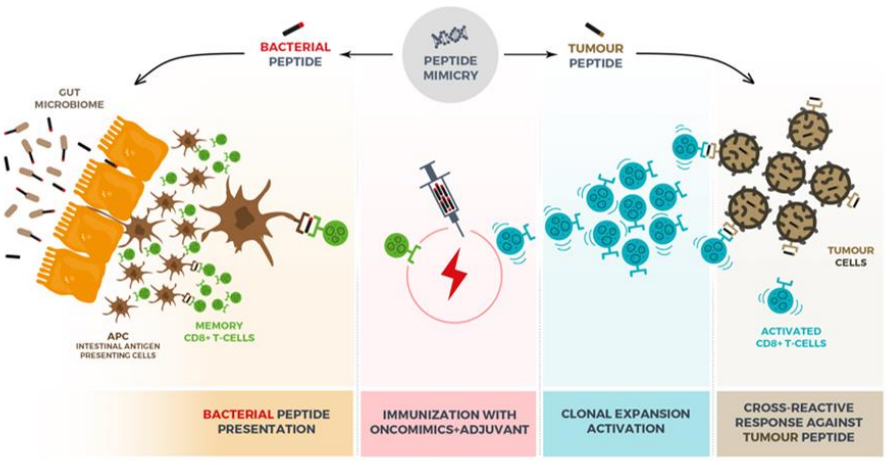
***In contrast to the analysis of tumor biopsy samples, which are not only invasive to obtain but often also do not fully capture tumor heterogeneity and evolution, the analysis of ctDNA offers a non-invasive method of longitudinally evaluating the genomic profile of a patient's cancer. Building on, among others, the above referenced study, data on the potential uses of ctDNA are rapidly accumulating in the continuum of care across multiple cancers.***

ctDNA is composed of short fragments of tumor-derived DNA (roughly 130-150 base pairs) that are released by cancer cells into the circulation via apoptosis, necrosis, or secretion. Detectable ctDNA after completion of curative therapies has nearly 100% positive predictive value for radiographic recurrence. Multiple studies demonstrate that the presence of ctDNA likely reflects radiographically unseen micrometastases, or MRD, which is predictive of recurrence and a useful screening tool for high-risk patients.

The lead-time of ctDNA positivity to radiographic recurrence is approximately 9 months in early-stage disease, which provides a window for evaluation of novel therapies in a setting for which there are no current standard-of-care guidelines.

***Clearance of ctDNA may be used as an endpoint in early-stage studies to stratify patients for more definitive trials and promote drug development in this space. The improved detection of ctDNA has provided a unique opportunity to use a noninvasive approach to explore therapeutic options and to potentially eradicate micrometastatic disease in high-risk patients.***

Extensive pre-clinical studies have been conducted to characterize the microbiome-derived mimicry therapeutic vaccine concept as outlined in the figure below, and two multi-peptide compounds have been evaluated in clinical trials (EO2401 in glioblastoma, adrenocortical carcinoma, and malignant pheochromocytoma/paraganglioma; EO2463 in follicular and marginal zone lymphoma).




EO2040, the microbiome-derived mimicry peptide mix for treatment of CRC, has been developed to maximize the ability to generate a strong immune response, by high affinity MHC (Major histocompatibility complex) class I binding-peptides (i.e. targeting presentation for $CD8^+$ cytotoxic T cells), high capacity to be recognized by the immune system by its non-self-nature, and possibility for fast expansion of T cells by the pre-existence of T cell clones already recognizing the mimicry peptides which can be efficiently reactivated by a vaccine boost leading to a durable immune response. The concomitant administration of EO2040 and an anti-PD1 blocking agent is utilized to optimize the possibility for tumor specific T cells to infiltrate and effectively function within the tumor environment.

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| | The two tumor targeting peptides in EO2040, i.e. EO2317 and EO2318, were found in the human microbiota, and are restricted to HLA-A2 expressed in 49% of the Caucasian population. The microbiome-derived peptides display high sequence homologies with two different TAAs: survivin also called baculoviral inhibitor of apoptosis repeat-containing protein 5 (BIRC5), and forkhead box M1 (FOXM1), respectively. These TAAs are highly expressed in CRC, but not in normal tissues. The two different TAAs were selected to overcome possible tumor heterogeneity and reduce tumor escape. The peptides demonstrated in in vitro and in vivo pre-clinical testing high MHC binding affinity, and ability to elicit strong immune responses as well as cross reactivity against the human corresponding peptides.<br><br>***The microbiome-derived therapeutic vaccine concept utilized in conjunction with anti-PD1 blockade is an innovative option for testing of a rational immunotherapy in CRC. The concept as such, including the combination with nivolumab, has already been tested in the clinical setting (i.e. in recurrent glioblastoma and adrenal tumors) and shown to be well tolerated, capable of expanding tumor target specific T cells very rapidly and extensively, and having the ability to shrink advanced solid tumors.***<br><br>***Based on the above, the current study will evaluate the microbiome-derived therapeutic vaccine EO2040 in combination with nivolumab in patients with circulating tumor DNA-defined MRD of colorectal cancer stage II, III, or IV after completion of standard curative therapy.*** |

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| **Objectives** | **Primary objective**<br>• The primary objective of this trial is to assess the 6-month ctDNA clearance rate at therapy with EO2040 in combination with nivolumab, in patients with ctDNA-defined MRD of stage II-IV colorectal cancer after completion of curative therapy.<br>*and in sequence, provided the first objective has a positive outcome (see Primary Endpoint):*<br>• 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.<br>*ctDNA clearance is utilized as a surrogate endpoint for eventual cure, and thereby prolongation of disease-free survival (DFS) for patients achieving clearance.*<br>*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.*<br>**Secondary objectives**<br>To assess the following:<br>• safety and tolerability of study treatments,<br>• the 3-month ctDNA clearance rate,<br>• progression of colorectal cancer and death as disease-free survival (DFS),<br>• overall survival (OS),<br>• survival at 36 months after start of study therapy, and<br>• induction/expansion of T cells specific for EO2040, the components of EO2040, and the targeted nominal TAAs (BIRC5 and FOXM1).<br>**Exploratory objectives**<br>1. [redacted]<br>2. [redacted]<br>3. 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.<br>4. [redacted] |

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| **Endpoints** | **Primary endpoint**<br><br>Response to therapy will be measured with a standard-of-care, CLIA certified ctDNA assay, or similar certification outside of the USA (timepoint 6 months).<br><br>Identification of somatic mutations in the blood will be confirmed by sequencing of the resected tumor using a CLIA-certified next-generation sequencing panel. 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.<br><br>The primary endpoint is response to treatment at 6 months, which is defined as having clearance of ctDNA, AND not having any radiographic evidence of recurrence at 6 months.<br><br>[redacted]<br><br>**Secondary endpoints**<br><br>1. 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.<br>2. 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 endpoints).<br>3. Disease-free survival (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.<br>4. Overall survival (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.<br>5. Survival at 36 months after start of study treatment, estimated via Kaplan-Meier estimates.<br>6. 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]<br><br>**Exploratory endpoints**<br><br>1. [redacted] |

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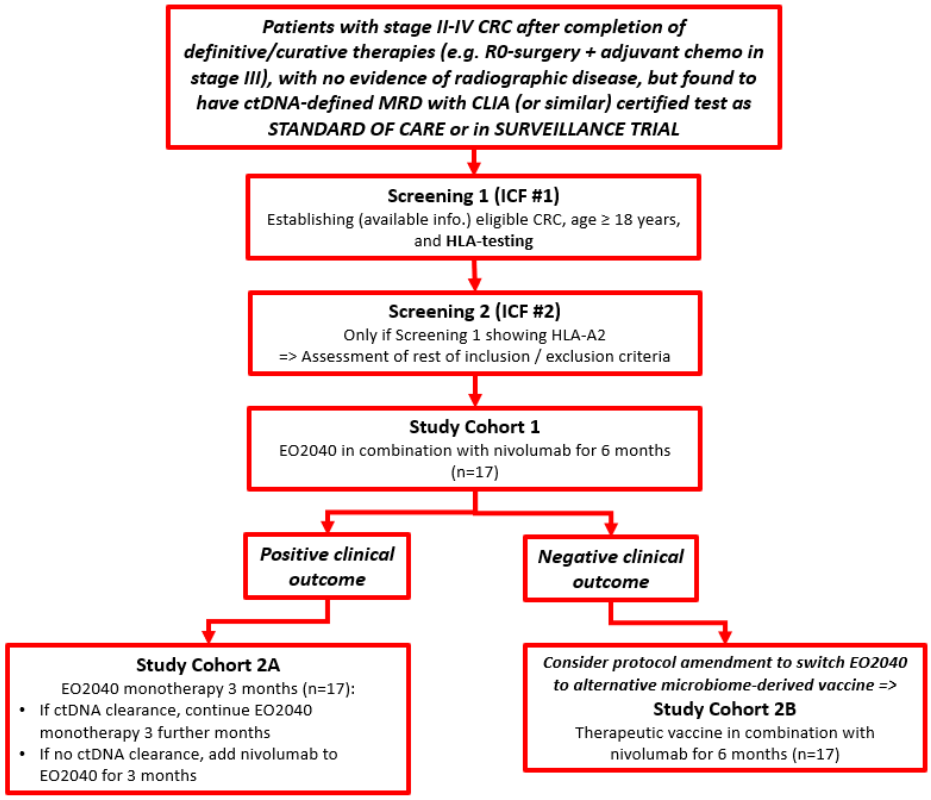
|---|---|
| | [redacted]<br>3. Correlations between immunogenicity [redacted] of EO2317, EO2318, and UCP2 and clinical efficacy (per primary and secondary efficacy endpoints) and safety (TEAEs of defined specificities and grades) outcome parameters. Cross reactivities shown for the TAAs BIRC5/survivin, and FOXM1 will also be explored in the same way.<br>[redacted] |
| **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 (see Primary Endpoint), 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.<br>The study is assumed to recruit a total of 17 patients in Cohort 1, and if Cohort 1 has a positive outcome, a total of 17 patients will also be recruited to Cohort 2A. Thus, provided a positive outcome of Cohort 1, a total of 34 patients are assumed to start study treatment.<br>Generally, no replacement is foreseen. [redacted] |

[redacted]

## Overview trial design




[redacted]

There will be a 2-stage consent procedure for the trial, including a first consent for basic disease, age information, and HLA-typing (or the use of already available information in the patients' file; such information even if based on testing before the screening period is

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| | acceptable) since a large proportion of the patients (approximately half of the patients) will not match the HLA-A2 prerequisite to receive the HLA-A2 specific EO2040 immunization compound.<br><br>The second stage of the consent procedure will be related to treatment, and follow-up aspects of the trial procedures.<br><br>[redacted]<br><br>Study treatments will be administered until study treatment termination criteria as outlined in Section 5.3.3 are fulfilled, e.g. reaching maximum study treatment period (6 months), unacceptable toxicity, or relapsing disease. At the time of stopping study treatment, appropriate other treatment will be advised by the treating physician on an individual patient basis. The patient should continue, even if study treatment is stopped, study follow-up measures for as long as the individual patient consent for follow-up is not withdrawn, the site is open, and the study not terminated per plan, or early, but for a maximum length of 3 years after start of study treatment.<br><br>All assessments of possible relapse in the trial will be determined at the sites by the principal investigators, or designees, according to the standard clinical criteria used at the site.<br><br>***Of note, there is no current knowledge publicly available regarding the potential of pseudoprogression at immunotherapy in patients with ctDNA defined MRD of CRC. Thus, at scanning, especially at the 3-month study timepoint, if a relapse is indicated by scanning it is recommended to, if at all possible, also document the relapse with histology or cytology.*** |
| **Visit schedule** | Section 7 of the trial protocol outlines study assessments and procedures, and their timings (including visit windows) in the Study Schedule. |
| **Inclusion/ exclusion criteria** | **Inclusion criteria**<br>To be eligible to receive study treatment, a patient must meet all the criteria below:<br>1. Provided written informed consent prior to any study-related procedures (initial consent is for screening part #1 procedures, and a second consent is for screening part #2 procedures; see Section 7.2 and Section 7.3, respectively).<br>2. Histological confirmation of colorectal cancer (confirmation at initial diagnosis is sufficient).<br>3. Post R0-resection (i.e. microscopically margin-negative resection and no gross or microscopic tumor remaining in the primary tumor bed) of stages II, III, or IV CRC and completion of all planned standard of care perioperative and/or adjuvant therapies.<br>*a.* [redacted]<br>4. Presence of minimal residual disease as defined by a positive ctDNA assay after completion of all planned standard of care therapies.<br>*a.* [redacted] |

[redacted]

5. Age ≥ 18 years old.
6. Human leukocyte antigen (HLA)-A2 positive.
7. No evidence of radiographic disease (computer tomography or magnetic resonance imaging; optimized to detect metastatic disease, e.g. with contrast as applicable) that requires immediate therapeutic intervention as assessed by the treating physician, within 28 days of (before or after) a positive ctDNA assay.

[redacted]

8. ECOG performance status 0 or 1 (see Section 12.1).
9. Female patients of childbearing potential must have a negative serum pregnancy test within 72 hours prior to start of study therapy.
10. Considering the embryofetal toxicity of the immune checkpoint inhibitor (ICI) shown in animals' models, the following recommendations for contraception must be followed:
    a. If not surgically sterile, female patients of childbearing potential age must use highly effective contraception from signing the Informed Consent Form (ICF) through 6 months after the last treatment dose administered. Highly effective contraception includes (according to Clinical Trial Facilitation Group: Recommendations related to contraception and pregnancy testing in clinical trials [104]):
        i. combined (estrogen and progesterone containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, transdermal,
        ii. progestogen-only hormonal contraception associated with inhibition of ovulation: oral, injectable, implantable,
        iii. intrauterine device (IUD),
        iv. intrauterine hormone-releasing system (IUS),
        v. bilateral tubal occlusion,
        vi. vasectomized partner, and
        vii. sexual abstinence when in line with the preferred and usual lifestyle of the patient (e.g. periodic abstinence is not considered a highly effective method).

    In each case of delayed menstrual period (over 1 month between menstruations), confirmation of absence of pregnancy is strongly recommended. This recommendation also applies to women of childbearing potential with infrequent or irregular menstrual cycles.

b. If not surgically sterile, male with female partner of childbearing potential age must use condom from signing the ICF through 6 months after the last treatment dose administered. Males must ensure that their partners of childbearing potential use highly effective contraception also. [redacted]

11. Patients willing and able to comply with the scheduled visits, treatment plan, laboratory tests, and other study procedures indicated in the protocol.

**Exclusion criteria**

Patients who meet any of the following criteria will not be eligible to participate in the study:

1. Patients treated with dexamethasone > 2 mg/day or equivalent (i.e., 13 mg/day of prednisone) within 14 days before start of study therapy, unless required to treat an adverse event (AE).

[redacted]

2. Patients treated with radiotherapy within 12 weeks, and cytotoxic chemotherapy therapy within 28 days (or 5 half-lives of the compound(s) administered if longer) before study treatment start.
3. Patients with persistent Grade ≥ 2 toxicities (according to NCI-CTCAE v5.0). Toxicities must be resolved for at least 2 weeks to Grade 1 or less. However, alopecia, neuropathy, and other persisting toxicities not constituting a safety risk based on Investigator's judgment are acceptable.
4. Patients who have received any prior treatment with compounds targeting PD1, PD-L1, CTLA-4, or similar compounds where general resistance against therapeutic vaccination approaches might have developed.
5. Patients with the following abnormal laboratory values:
    a. Hemoglobin < 10 g/dL (6.2 mmol/L); transfusion is acceptable to reach the value.
    b. Absolute neutrophil count decrease (<1.5 x$10^9$/L).
    c. Platelet count decrease (< 75 ×$10^9$/L).
    d. Total bilirubin > 1.5 ×upper limit of normal (ULN; according to the performing laboratory's reference ranges); except participants with Gilbert Syndrome who must have a total bilirubin level of < 3.0 xULN.
    e. Alanine aminotransferase (ALT) > 3 ×ULN.
    f. Aspartate aminotransferase (AST) > 3 ×ULN.
    g. Serum creatinine increase (> 1.5 ×ULN).
    h. Abnormal thyroid function per local laboratory levels; note, patients with hypothyroidism only requiring hormone replacement therapy, and patients with long term (judged by the treating physician) stable antithyroid therapy due to hyperthyroidism, are permitted to enroll. Patients with abnormal thyroid laboratory values judged by the treating physician as clinically non-relevant are also eligible.

6. Patients with presence of other concomitant active, invasive malignancies that may interfere with ctDNA analysis (known clonal hematopoiesis of unknown potential allowed).
7. Patients with clinically significant active infection, cardiac disease, significant medical or psychiatric disease/condition that, in the opinion of the Investigator, would interfere with the interpretation of patient safety or study results or that would prohibit the understanding or rendering of informed consent (i.e. only consent able patients can be enrolled in the study) and compliance with the requirements of the protocol – including (but not limited to):
    a. Bacterial sepsis, COVID-19, or other similarly severe infections (clinical assessment is the basis for exclusion of severe infections; if clinical suspicion, adequate testing should be performed to exclude severe infections).
    b. New York Heart Association > Grade 2 congestive heart failure within 6 months prior to start of study therapy (see Section 12.2).
    c. Uncontrolled or significant cardiovascular disease, including:
        i. myocardial infarction within 6 months prior to start of study therapy,
        ii. uncontrolled/unstable angina within 6 months prior to start of study therapy,
        iii. diagnosed or suspected congenital long QT syndrome, and
        iv. any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes).
    d. Stroke within 6 months prior to start of study therapy.
    e. Concurrent neurodegenerative disease.
    f. Dementia or significantly altered mental status.
8. Patients with suspected autoimmune or active autoimmune disorder or known history of an autoimmune neurologic condition (e.g., Guillain-Barré syndrome).

[redacted]

9. Patients with a history of solid organ transplantation or allogeneic hematopoietic stem cell transplantation.
10. Patients with a history or known presence of tuberculosis.
11. Pregnant and breastfeeding patients.
12. Patients with a history or presence of human immunodeficiency virus (HIV) and/or active hepatitis B virus (HBV)/hepatitis C virus (HCV).
13. Patients who have received live or attenuated vaccine therapy used for prevention of infectious diseases including seasonal (influenza) vaccinations within 4 weeks of the first dose of study drug.
14. Patients with a history of hypersensitivity to any excipient, or active substance, present in the pharmaceutical forms of applicable study treatments.

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| | 15. Patients under treatment with immunostimulatory or immunosuppressive medications, including herbal remedies, or herbal remedies known to potentially interfere with major organ function.<br>16. Patients who have received treatment with any other investigational agent, or participation in another clinical trial (clinical trial including active interventions; participation in clinical trials for data collection purposes only are permitted) within 28 days (or 5 half-lives if longer) prior to start of study therapy. Note, during participation in the current study, parallel participation in other interventional studies is not allowed (participation in clinical trials for data collection purposes only are permitted; also, in the follow-up parts of interventional studies). |
| **Investigational and standard of care treatments** | **Investigational treatments**<br>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) UCP2.<br>The peptide mix EO2040, i.e. drug product (DP), [redacted] subcutaneous (SC) administration.<br>EO2040 will be 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 will also be evaluated as monotherapy.<br>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 will follow the current version of European SmPC and US PI.<br>**General standard of care treatments**<br>All non-cancer therapies that the Investigator feels appropriate are allowed in this study, except for the medications outlined in protocol Section 6.7.3. Thus, patients should receive full supportive care during participation in the trial and standard of care treatment per local practice and according to the judgment of the Investigator or treating physician.<br>The use of systemic corticosteroids and other immunosuppressants (beside trial treatment) should be avoided because of their potential interference with the pharmacodynamic activity of the investigational treatment. However, the use of systemic corticosteroids and other immunosuppressants might be of vital importance for patient safety in relation to treatment of adverse reactions. Further guidance regarding steroid treatment can be found in the trial protocol Section 6.7.2. |
| **Independent Data Monitoring Committee (IDMC)** | The IDMC will serve as an external monitoring group for the study. The primary role of the IDMC will be to examine the safety and tolerability of study participants throughout the duration of the study. The IDMC will be created to further protect the rights, safety, and well-being of patients who will be participating in the trial by monitoring their progress and results.<br>[redacted] |

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| | [redacted] |
| **Statistical considerations** | **Statistical methods**<br><br>All data collected in this trial will be reported by patient data listings, summary tables, and figures for all demographic and baseline characteristics, medical history, efficacy, and safety variables. Proportions and the denominators will be provided to summarize response, toxicity, and other categorical variables.<br><br>The response rate R6 will be estimated using all patients in the FAS with response at 6 months in the numerator and all patients in the FAS in the denominator; [redacted]<br><br>Statistical assessments of correlations between e.g. immunogenicity parameters and efficacy and safety outcome parameters will be conducted as described in the Statistical Analysis Plan (SAP).<br><br>The SAP will provide all details with respect to patients being analyzed for all endpoints. Relevant subgroups based on available data and compliance to protocol procedures might be defined.<br><br>Statistical tests may be planned in the SAP or performed as needed. The importance of the tests does not reside in making conclusions on statistical relevance of differences, but rather identification of possible results that could be worth further exploration.<br><br>**Determination of sample size & primary analysis**<br><br>This is an early development, open-label, exploratory, trial to include patients with CRC who have early disease which today is without an established standard of care, but still in a relevantly early stage of the overall disease history that there is a potential chance to significantly delay recurrence/progression with an efficacious therapy. Thus, a balance needs to be struck between a size to find a relevant signal, and the importance to not expose too many patients to a possibly non-efficacious therapy.<br><br>Considering prior attempts in similar situations, the trial will include 17 patients per cohort. Utilizing FAS as the population for the primary analysis, [redacted]<br><br>The primary objective of the trial is to determine the ctDNA clearance rate at 6 months (R6).<br><br>[redacted]<br><br>For the primary analysis, the ctDNA clearance rate will be estimated along with the 95% exact confidence interval. The efficacy of study treatment will also be assessed by performing a binomial test comparing the ctDNA clearance rate against the null hypothesis 5%, at a one-sided alpha level of 0.025. |

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| | [redacted]<br><br>[redacted]<br><br>The opening of a Cohort 2A exploring EO2040 monotherapy (with the option of addition of nivolumab if ctDNA is not cleared at 3 months) is dependent on the outcome of Cohort 1; the main factor being the assessment of ctDNA clearance rate (which should be positive per above), [redacted]. If no continuation of exploration of EO2040 would be seen as appropriate based on data from Cohort 1, there will be considerations regarding a protocol amendment to switch EO2040 to an alternative microbiome-derived vaccine.<br><br>Based on the above, it is assumed that the current trial will include enough patients to provide safety and tolerability information, immunogenicity data, as well as preliminary efficacy data in the selected treatment setting without demanding a too high (and long) patient recruitment and without exposing too many patients. |
| **Interim analyses** | The nature of the study, i.e. early exploratory development trial aiming at generating as much knowledge as possible before potential decisions related to further development of EO2040, makes it important to assess especially safety, but also efficacy and possible biomarkers, on an ongoing basis during trial conduct. Exploratory preliminary data from the trial might be utilized in relation to e.g. scientific discussions and presentations to facilitate input regarding possible improvements of development parameters.<br><br>[redacted] |
| **Number of patients, sites, location, and predicted durations** | **Number of patients**<br>• The study is to recruit 34 patients (17 patients in each of two sequential cohorts).<br>**Locations and number of sites**<br>• USA, 2-3 sites, and Germany, 3-5 sites<br>**Recruitment time, max study therapy time, and follow-up time**<br>• [redacted]<br>[redacted]<br>[redacted]<br><br>[redacted] |

# 1 BACKGROUND

## 1.1 Colorectal cancer

### *1.1.1 Epidemiology, risk factors, and symptoms*

Colorectal cancer (CRC) is the third most common tumor in men and the second in women, accounting for 10% of all tumor types worldwide. Incidence is 25% higher in males and differs greatly between countries. With more than 600 000 deaths estimated each year, CRC is the fourth most common cancer-related cause of death globally [11].

In 2020, an estimated 104,610 new cases of colon cancer and 43,340 cases of rectal cancer will occur in the USA [2]. During the same year, an estimated 53,200 people will die of colon and rectal cancer combined. Despite these high numbers, the incidence of colon and rectal cancers per 100,000 people decreased from 60.5 in 1976 to 46.4 in 2005, and more recently, 38.7 in 2016 2].

The mortality rate in the European Union is 15-20 out of 100,000 in males and 9-14 out of 100,000 in females and has decreased over time, particularly in females. In affected European individuals, 5-year survival ranges from 28.5% to 57% in men and from 30.9% to 60% in women, with a pooled estimation in 23 countries of 46.8% in men and 48.4% in women [11].

The risk of developing colon cancer depends on factors which can be classified into lifestyle or behavioral characteristics and genetically determined factors. Age is considered the major unchangeable risk factor for sporadic colon cancer: nearly 70% of patients are >65 years of age and this disease is rare before the age of 40 years, even though data from Western registries show an increased incidence in the 40- to 44-year age group 1Individuals with any of the following are considered at high risk of colon cancer and must be actively screened and in case of inherited syndromes, also referred for genetic counselling:

- a medical history of adenoma, colon cancer, inflammatory bowel disease (Crohn's disease and ulcerative colitis),
- significant family history of CRC or adenoma,
- an inherited cancer syndrome (2%-5% of all CRC), such as familial adenomatous polyposis coli and its variants (1%), Lynch-associated syndromes (hereditary non-polyposis colon cancer) (2%-4%), Turcot, Peutze-Jeghers, and MUTYH-associated polyposis syndrome.

Colon cancer arises from the mucosa of the bowel, growing both into the lumen and the bowel wall, and/or spreading to adjacent organs. Symptoms are associated with relatively large tumors and/or advanced disease stages and may not be specific for colon cancer. Alterations in bowel habit, general or localized abdominal pain, weight loss without other specific causes, weakness, iron deficiency and anemia are the most common symptoms and depend on the location and stage of the primary tumor [2].

### *1.1.2 Curative treatment settings*

After appropriate diagnostic measures surgical intervention is the first key step in treatment of colon cancer [1, 2].

Assuming surgery for cure, i.e. R0-resection with microscopically margin-negative resection and no gross or microscopic tumor remaining in the primary tumor bed, the assessment of risk of recurrence is important in deciding when to recommend systemic adjuvant treatment with the aim of reducing risk of relapse and death. The risk of relapse after colon cancer resection is estimated by integrating the clinicopathological features of the tumor with the molecular marker mismatch repair (MMR)/microsatellite instability (MSI) status [1].

TNM staging remains the most relevant histological criteria for risk assessment after surgery of colon cancer. Reported 5-year survival rates after surgical resection alone are 99% for stage I, 68%-83% for stage II and 45%-65% for stage III disease [1]. In addition, for intermediate stage II, further parameters need consideration to refine the evaluation of risk given the observed variability on prognosis [1].

In general, it has been established that adjuvant systemic therapy decreases the risk of death by an absolute 3%-5% in high-risk stage II colon cancer with single-agent 5-fluorouracil (5-FU) and by 10%-15% in stage III disease with fluoropyrimidines alone, with a further 4%-5% improvement with oxaliplatin-containing combinations [1].

FOLFOX (leucovorin/5-fluorouracil/oxaliplatin) and CAPOX (capecitabine plus oxaliplatin) remain the current standard of care adjuvant treatments for stage III disease. Note, irinotecan, cetuximab and bevacizumab have not demonstrated clinical activity in the localized disease setting and therefore they should never be used as adjuvant treatment in this setting [1]. Learned societies gives further guidance on treatments to use, or not to use, in specific stages of disease and length of treatments which are preferable from a benefit/risk perspective [1, 2, 5].

Studies of selected patients undergoing surgery to remove CRC liver metastases have shown that cure is possible in this population and should be the goal for a substantial number of these patients [2]. Reports have shown 5-year disease-free survival (DFS) rates of approximately 20% in patients who have undergone resection of liver metastases, and a recent meta-analysis reported a median 5-year survival of 38% [2]. In addition, retrospective analyses and meta-analyses have shown that patients with solitary liver metastases have a 5-year overall survival (OS) rate as high as 71% following resection [2].

### *1.1.3 Surveillance after curative treatments*

The NCCN panel recommendation for post-treatment surveillance for patients with stage II/III disease who have undergone successful treatment (i.e. no known residual disease) include the following procedures [2]:

- History and physical examination should be given every 3 to 6 months for 2 years, and then every 6 months for a total of 5 years.
- A carcinoembryonic antigen (CEA) test is recommended at baseline and every 3 to 6 months for 2 years, then every 6 months for a total of 5 years for patients with stage III disease and those with stage II disease if the clinician determines that the patient is a potential candidate for aggressive curative surgery.
- Colonoscopy is recommended at approximately 1 year after resection (or at 3-6 months post-resection if not performed preoperatively because of an obstructing lesion). Repeat colonoscopy is typically recommended at 3 years, and then every 5 years thereafter, unless follow-up colonoscopy indicates advanced adenoma, in which case colonoscopy should be repeated in 1 year.
- Chest, abdominal, and pelvic CT scan are recommended every 6 to 12 months for up to 5 years in patients with stage III disease and those with stage II disease at a high risk of recurrence.

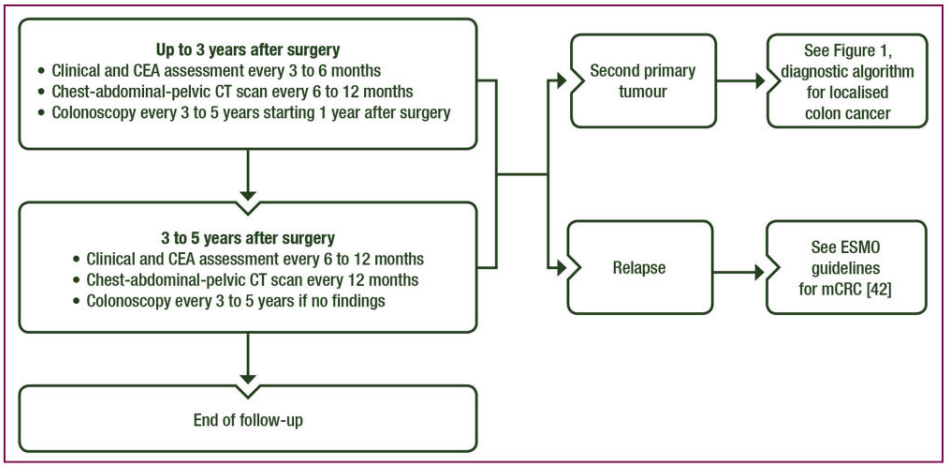
The NCCN panel recommendation for surveillance of patients with stage IV CRC with no evidence of disease (NED) after curative-intent surgery and subsequent adjuvant treatment are similar to those listed for patients with stage II/III disease, except that certain evaluation are performed more frequently. The NCCN panel recommends that these patients undergo contrast-enhanced CT scan of the chest, abdomen, and pelvis every 3 to 6 months in the first 2 years after adjuvant treatment and then every 6 to 12 months for up to a total of 5 years [2]. CEA testing is recommended every 3 to 6 months for the first 2 years and then every 6 months for a total of 5 years, as for patients with earlier stage disease [2].

Patients with an elevated CEA level after resection should be investigated with chest, abdominal and pelvic CT scan, and physical examination, and consideration of a PET/CT-scan. If imaging study results are normal in the face of a rising CEA, repeat CT scans are recommended every 3 months until either disease is identified, or CEA level stabilizes or declines [2].

The ESMO guideline regarding localized colon cancer is in principle similar to the NCCN panel recommendations for stage II/III disease (**Figure 1** [1]). However, the ESMO guidelines does not give more specific recommendations regarding surveillance after potentially curative surgery of stage IV disease, or at an increase of CEA during surveillance [1, 3].

**Figure 1 : ESMO clinical practice guidelines for localized colon cancer; recommendations for follow-up after curative resection.**

***CEA, carcinoembryonic antigen; CT, computed tomography; mCRC, metastatic colorectal cancer.***




### *1.1.4 Metastatic disease*

Despite all efforts regarding surgery and adjuvant therapy per above, approximately 25% of patients who present with localized disease will later develop metastases [3]. In addition, of new colorectal cancer diagnoses, 20% of patients have metastatic disease already at presentation [3].

The above numbers are consistent with the estimates presented by NCCN, that approximately 50%-60% of patients diagnosed with CRC develop metastatic disease, and 80%-90% of these patients have unresectable metastatic liver disease [2]. Also, that metastatic disease most frequently develops metachronously after treatment for locoregional CRC, with the liver being the most common site of involvement, and that 20%-34% of patients with CRC present with synchronous liver metastases [2].

Metastatic disease not amenable to local treatment only (e.g. surgery, stereotactic body radiation therapy, haptic arterial infusion, arterial embolization therapy, tumor ablation via for instance radiofrequency ablation or cryotherapy and other means), is usually treated with systemic chemotherapy or targeted treatments as outlined in different guidance documents [1, 2].

According to the NCCN guideline, recommendations for patients with disseminated metastatic disease represent a continuum of care in which lines of treatment are blurred rather than discrete [2]. In addition to fluoropyrimidine-, oxaliplatin-, and/or irinotecan-containing chemotherapy regimens, immunotherapy and targeted therapy regimens are becoming an increasingly important part of the treatment landscape for patients with metastatic CRC. Combination of a biologic agent (e.g. bevacizumab, cetuximab, panitumumab) with some of the chemotherapy regimens is an option, depending on available data. Systemic therapy

options for patients with progressive disease depend on the choice of initial therapy and biomarker status of the tumor.

As outlined in the ESMO guidance regarding metastatic CRC [4], a patient with classical metastatic CRC may typically achieve an OS of approximately 30 months as the result of a multidisciplinary team-managed 'continuum of care'. An example of a typical 'continuum of care' treatment sequence is outlined below:

- approximately 4–6 months of first-line 'induction' therapy,
- 4–6 (–8) months of 'maintenance' therapy - or no treatment after resection and/or ablation following first-line treatment,
- about 3 months re-introduction (or treatment beyond progression)
- 5–7 months of second-line therapy,
- a treatment break before initiation of a further line,
- approximately 3 months of third-line therapy,
- potentially a fourth line (in patients with RAS wild-type disease),
- a few months of re-challenge of initial induction or first-line therapy, and
- a few months best supportive care only.

Among people diagnosed with metastatic colorectal cancer, approximately 70%-75% of patients survive beyond 1 year, 30%-35% beyond 3 years, and fewer than 20% beyond 5 years from diagnosis [3].

***Thus, CRC continues to be a major therapeutic challenge with a considerable number of patients experiencing premature death from early disease recurrence. Historically, it has been challenging to definitively identify patients with CRC at risk of recurrence after completed assumed curative treatment, which has complicated the ability to optimize adjuvant drug development. Relapsed CRC may occur in the absence of symptoms, further emphasizing the importance of sensitive screening modalities.***

## 1.2 Minimal residual disease setting in colorectal cancer

Wan et al. has published a thorough review regarding liquid biopsies for residual disease and recurrence, covering the background necessary for current trial [6].

### *1.2.1 Circulating tumor DNA*

Circulating fragments of cell-free DNA were first described in 1948 by Mandel and Métais [7]. Proof-of-principle studies demonstrated the potential clinical utility of detecting circulating tumor DNA (ctDNA) in the 1990s. For example, DNA fragments carrying mutations in the KRAS gene were identified in the plasma of patients with pancreatic cancer using allele-specific polymerase chain reaction (PCR) [8]. Multiple studies, using sensitive mutation analysis in plasma of cancer patients and xenograft models, confirmed that ctDNA fragments are derived from cancer cells and could be used as a quantitative marker to assess cancer disease burden [9, 10, 11]. In parallel, the introduction of massively parallel or next-generation sequencing (NGS) has enabled an astonishing progress in cancer genomics from both tissue and body fluid samples [12].

ctDNA can be detected at varying concentrations across multiple cancer types and disease stages [13]. ctDNA typically constitutes a small proportion of an individual's total circulating cell-free DNA, <1% according to some studies. However, with improving assay techniques providing greater levels of sensitivity, the analysis of ctDNA is rapidly being accepted as a reliable tool in oncology. The proportion of patients in whom ctDNA can be detected depends on the extent of tumor volume. For instance, in patients with CRC, the proportion of patients with detectable ctDNA ranges from 50% in those with non-metastatic disease to nearly 90% in patients with metastatic disease.

In patients with advanced-stage disease, where levels of ctDNA are higher, detection of genomic alterations in specific genes by analysis of plasma samples can be used to guide targeted therapies, and analysis of ctDNA can be used to assess for instance overall mutation rates (potential to inform on the likelihood of efficacy of immunotherapies), and response to treatment or identify progression (fractional concentration monitored over time) [6].

An important example of the use of personalized ctDNA analysis as a predictive biomarker in patients with solid tumors treated with immune checkpoint blockade (pembrolizumab) was recently published by Bratman et al [14]. The study investigators applied ctDNA assays to 316 serial plasma samples obtained at baseline and every three cycles from 94 patients; baseline ctDNA concentration correlated with progression-free survival, overall survival, clinical response and clinical benefit. This association became stronger when considering ctDNA kinetics during treatment. Of note, all 12 patients with ctDNA clearance during treatment were alive with median 25 months follow up. According to the authors "This study demonstrates the potential for broad clinical utility of ctDNA-based surveillance in patients treated with immune checkpoint blockade".

For patients with early-stage solid tumors, ctDNA testing may identify patients who have residual disease or recurrence after potentially curative treatments (surgery in particular) [15, 16, 17, 18, 19]. On the basis of this evidence, multiple clinical trials have been initiated to evaluate the clinical validity and utility of this approach [6].

An analysis from the large GALAXY study, an observational study monitoring MRD, evaluating the association of ctDNA dynamics with a short-term clinical outcome and adjuvant chemotherapy efficacy was recently presented [23]. A total 1,365 patients with CRC were enrolled between June 2020 and April 2021 and included in the analysis; 116 stage I, 478 stage II, 503 stage III, and 268 oligometastatic resectable stage IV. The analysis demonstrated the association of ctDNA dynamics with improved clinical outcomes in MRD+ patients; also showing that stratifying post-surgical treatment decisions using a ctDNA assay can identify patients likely to benefit from adjuvant chemotherapy across all stages, including stage II [23].

***In contrast to the analysis of tumor biopsy samples, which are not only invasive to obtain but often also do not fully capture tumor heterogeneity and evolution, the analysis of ctDNA offers a non-invasive method of longitudinally evaluating the genomic profile of a patient's cancer. Building on, among others, the above referenced studies, data on the***

***potential uses of ctDNA are rapidly accumulating in the continuum of care across multiple cancers.***

### *1.2.2 ctDNA-defined minimal residual disease*

ctDNA is composed of short fragments of tumor-derived DNA (roughly 130-150 base pairs) that are released by cancer cells into the circulation via apoptosis, necrosis, or secretion [20]. Detectable ctDNA after completion of curative therapies has nearly 100% positive predictive value for radiographic recurrence. Multiple studies demonstrate that the presence of ctDNA likely reflects radiographically unseen micrometastases, or minimal residual disease (MRD), which is predictive of recurrence and a useful screening tool for high-risk patients [21].

The lead-time of ctDNA positivity to radiographic recurrence is up to 9 months in early-stage disease, which provides a window for evaluation of novel therapies in a setting for which there are no current standard-of-care guidelines [22].

***Clearance of ctDNA may be used as an endpoint in early-stage studies to stratify patients for more definitive trials and promote drug development in this space. The improved detection of ctDNA has provided a unique opportunity to use a noninvasive approach in order to explore therapeutic options and to potentially eradicate micrometastatic disease in high-risk patients.***

### *1.2.3* [redacted]

[redacted]

### 1.3 EO2040 a microbiome-derived therapeutic vaccine for treatment of minimal residual disease of colorectal cancer

[redacted]

Extensive pre-clinical studies have been conducted to characterize the microbiome-derived mimicry therapeutic vaccine concept (**Figure 2**), currently including two multi-peptide compounds in clinical trials (EO2401 in glioblastoma, adrenocortical carcinoma, and malignant pheochromocytoma/paraganglioma, and EO2463 in follicular and marginal zone lymphoma), and one compound in preparation for clinical trials (EO4010; targeted for multiple solid tumor types, including breast, colorectal and non-small cell lung cancer).

EO2040, the compound to be used in the current trial is a derivative of the compound EO2401. EO2040 include the same components as EO2401, except the microbiome derived peptide EO2316, mimicking an epitope on the TAA interleukin 13 receptor alpha-2 (IL-13Rα2). The reason to exclude peptide EO2316 from compound EO2040 is that IL-13Rα2 currently does not have a rationale to be used when colorectal cancer is targeted, i.e. expression levels as known today are not high enough.

Note, the parent compound of EO2040, i.e. EO2401, in combination with nivolumab, has been thoroughly investigated in two phase 1/2 trials in more than 100 patients with glioblastoma and adrenal tumors, showing the ability to generate strong systemic immune responses against the targeted TAAs, and being well tolerated; the safety profile being consistent with the profile of the combination partner nivolumab, with the only addition of local administration site reactions (most commonly grade 1, erythema, induration, and sometimes pain).

The microbiome-derived mimicry concept builds on utilization of peptide-based vaccination, an immunotherapeutic approach for the treatment of cancer that aims to deliver immunogenic peptides corresponding to specific tumor associated antigens (TAAs) to patients. The goal is to target the patient's antigen presenting cells (APCs), especially the dendritic cells (DCs), to induce efficient presentation of cancer epitopes to T lymphocytes that in turn leads to a sustained immune response against cancer cells expressing the targeted antigens at the tumor site. In the past, despite promising pre-clinical results in animal models, the cancer vaccination approach has not demonstrated unequivocal efficacy in patients. The lack of efficacy may be related to several factors including the status of the patient's immune system, the efficacy and specificity of antigen delivery, the lack of ability of T lymphocytes to infiltrate the tumor microenvironment and the immunosuppressive environment, for instance due to the expression of inhibitory checkpoint molecules. The ability of tumor antigens to generate a strong immune response depends on several factors including the affinity for the MHC class I or MHC class II complexes, the capacity of the antigen to be recognized by the immune system as self or non-self, and pre-existence of T-cell clones that can be efficiently reactivated by a vaccine boost.

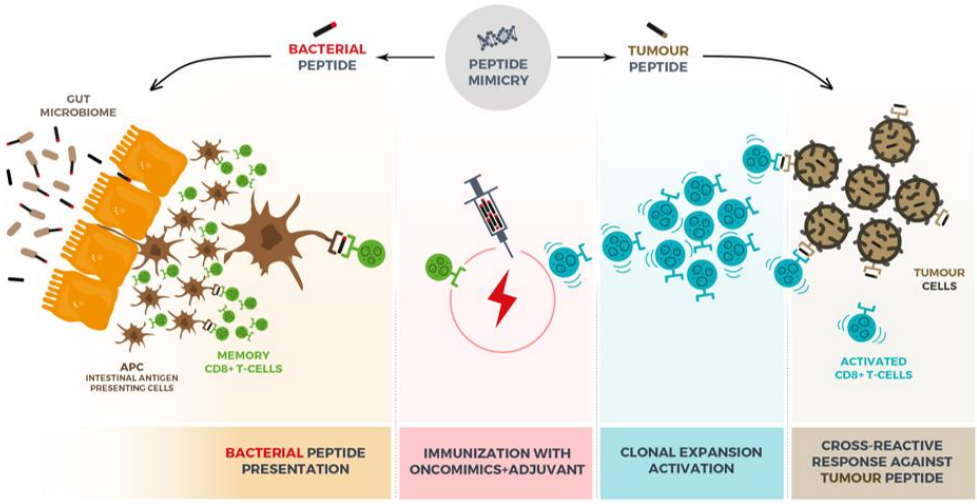
EO2040, the microbiome-derived mimicry peptide mix for treatment of minimal residual disease of colorectal cancer, was developed to maximize the ability to generate a strong immune response, by:

- high affinity MHC class I binding-peptides (i.e. targeting efficacious presentation for $CD8^+$ cytotoxic T cells),
- high capacity to be recognized by the immune system by its non-self-nature, and
- possibility for fast expansion of T cells by the pre-existence of T cell clones already recognizing the mimicry peptides which can be efficiently reactivated by a vaccine boost leading to a durable immune response.

In addition, the "off-the-shelf" approach minimize treatment delays due to need of producing patient specific vaccines.

The concomitant administration of EO2040 and an anti-PD1 blocking agent is utilized to optimize the possibility for tumor specific T cells to infiltrate and effectively function within the tumor environment (see further Section 1.3.5).

**Figure 2 : Microbiome-derived peptide therapeutic vaccine concept**




EO2040 Drug Product (DP; not adjuvanted) is a water/dimethyl sulfoxide (DMSO) peptide mixture solution of two synthetic [redacted] microbiome-derived peptides (EO2317 and EO2318), and the synthetic 15-amino acid helper peptide (Universal Cancer Peptide 2, UCP2). The peptide mix EO2040 (final concentration of each peptide is 300 µg/mL), [redacted] subcutaneous (SC) administration. EO2040 will also be given in combination with the anti-PD1 monoclonal antibody nivolumab delivered according to global labels.

The EO2317 and EO2318 peptides were found in the human microbiota, are restricted to HLA-A2 expressed in 49% of the Caucasian population [24] and display high sequence homologies with two different TAAs: survivin also called baculoviral inhibitor of apoptosis repeat-containing protein 5 (BIRC5), and forkhead box M1 (FOXM1), respectively.

These TAAs are highly expressed in colorectal cancer. The use of two different TAA targets were selected to overcome possible tumor heterogeneity and reduce tumor escape; both peptides demonstrated high MHC binding affinity, strong immune responses as well as cross reactivity against the human corresponding peptides in nonclinical models.

### *1.3.1 EO2317 and BIRC5/survivin*

EO2317 is a [redacted] microbial peptide with homology to a cognate T-cell epitope on BIRC5. *In vitro* binding assays showed high binding affinity of EO2317 for HLA-A2. [redacted]

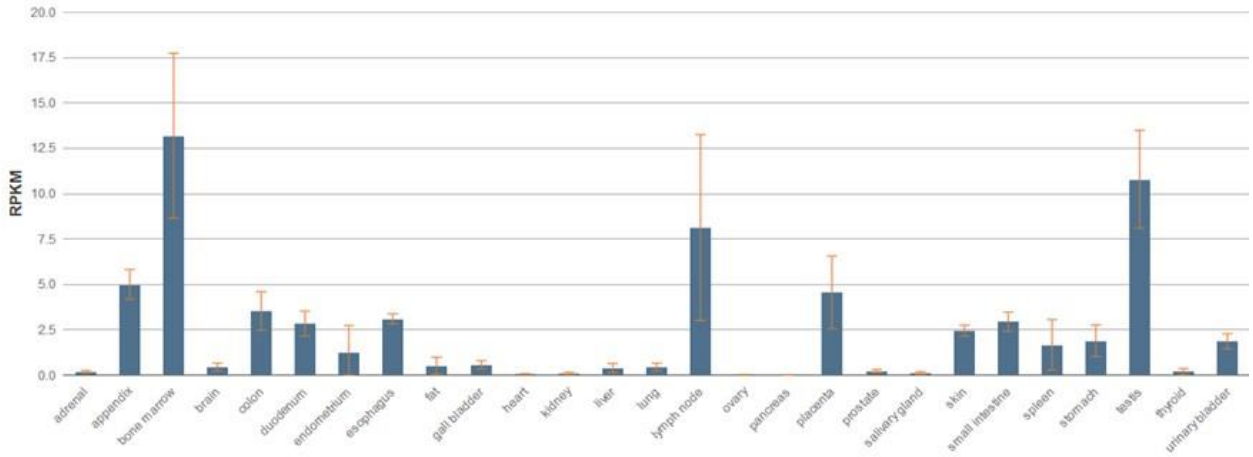
BIRC5, a well-known cancer driver gene, is a member of the inhibitor of apoptosis (IAP) gene family, which encodes negative regulatory proteins that function as endogenous inhibitors of caspases preventing apoptotic cell death [25]. BIRC5 also has a role as a mitosis regulator, physically associated with the mitotic apparatus thereby ensuring the proper completion of various stages of cell division probably via the regulation of microtubule dynamics and stability [26]. BIRC5 expression is high during fetal development and in most tumors, and absent to low in almost all adult tissues.

***BIRC5 Expression in normal human tissues (see* Figure 3*):***

While BIRC5 is strongly expressed in embryonic tissues where it plays a role in development, expression of BIRC5 in normal adult tissue is considered to be scarce. BIRC5 expression is developmentally regulated and has been reported to be low expressed in most terminally differentiated normal human tissues [27]. More recent studies using more sensitive methods have however revealed that some adult tissues could express BIRC5 albeit at levels lower than cancer cells. In adult tissues, BIRC5, known to regulate adult stem cell physiology, is expressed, and regulated in normal tissues characterized by self-renewal and proliferation as in hematopoietic stem cells, keratinocytes stem cells or intestinal stem cells [28].

Analysis of gene expression databases shows moderate expression of BIRC5 in testis and thymus with sometimes also low expression in lymph nodes, colon, and esophagus [29, 30, 31]. Massive peptide sequencing does not allow to detect specific BIRC5 peptides in normal tissues [32]. Other large-scale data available today on protein expression highlight the preferential expression of BIRC5 in the testis, with a low expression in the skin, oral mucosa, esophagus, and lymph nodes [31].

**Figure 3 : Relative BIRC5 mRNA expression in normal tissues; (HPA RNA-seq normal tissues [29]; RPKM: Reads Per Kilobase Million) RNA-seq performed on tissue samples from 95 human individuals representing 27 different tissues**



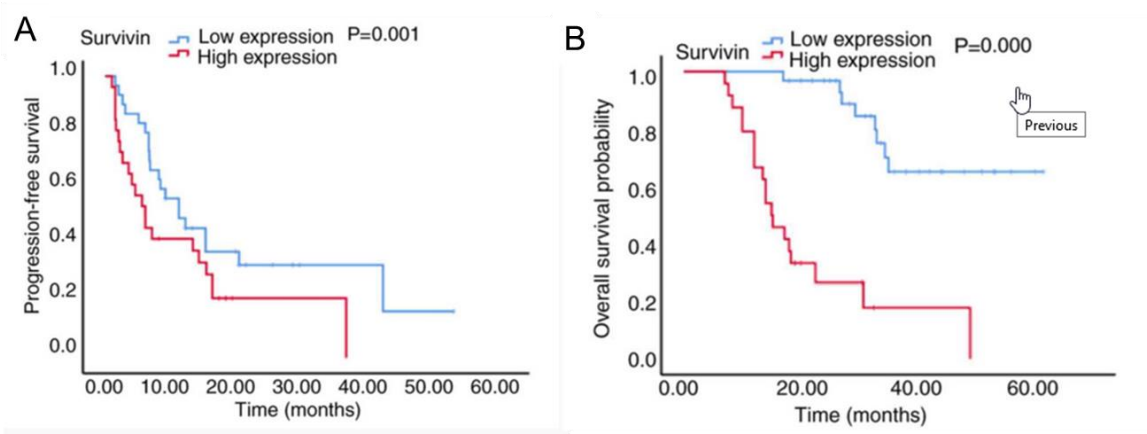

***BIRC5 Overexpression in colorectal tumor:***

As being able to control and regulate several pathways linked to tumorigenesis including protection from apoptosis and cell cycle promotion, BIRC5 is currently considered as a major tumor driver [33]. BIRC5 molecular functions are supported by a number of *in vitro* and *in vivo* preclinical studies that demonstrated that modulation of BIRC5 expression reduced tumor-growth, increased apoptosis and sensitized tumor cells to chemotherapeutic drugs [34]. Overexpression of BIRC5 in cancer overcome an apoptotic checkpoint and favour aberrant progression of transformed cells through mitosis.

BIRC5 has been defined as a universal tumor antigen. BIRC5 gene expression profiles across tumor samples and paired normal tissues (from the TCGA and the GTEx projects), highlight the clear overexpression of BIRC5 in many tumor types, including glioblastoma, colon adenocarcinoma, lung, or breast cancer [35]. Studies presented in the literature studies also demonstrate overexpression of BIRC5 in almost all type of neoplasms, including lung, colon, breast, pancreas, stomach, liver, ovary and prostate cancer, melanoma, hematopoietic malignancies, and glioma [36, 37].

In colon cancer, expression of BIRC5 is clearly documented. BIRC5 shows no or very low expression in normal colon tissues while BIRC5 is expressed in colon polyps and overexpressed in colonic adenocarcinoma [38]. Overexpression is observed at both the mRNA and protein levels with overexpression often seen in 80% to 100% of analyzed samples [38, 39, 40, 41]. Survivin expression in colon cancer is correlated with the degree of differentiation, depth of invasion, lymph node metastasis and Duke's stage [42, 43], and is associated with poor prognosis (**Figure 4**) [43, 44]. Interestingly, BIRC5 overexpression is also described in colorectal cancer stem cells [108, 109], and even proposed as a biomarker to characterize circulating tumor cells and predict OS in mCRC patients [110, 111].

**Figure 4 : Kaplan-Meier survival curves for PFS (A) and OS (B) in patients with stage IV colorectal cancer (N=71) [44]**




Overall, the large pattern of overexpression of BIRC5 in tumor, associated with its critical role in tumorigenesis makes BIRC5 an interesting chemotherapy 45], and immunotherapy target [47]. BIRC5 expression was shown to be significantly correlated with multiple immune cells infiltrates in a variety of tumor, and thus proposed as prognostic biomarker associated with tumor immune cell infiltration [37]. Furthermore, detection of BIRC5 specific T-cells in peripheral blood from patients with melanoma, lymphoma [47], glioma [48], and colorectal cancer [49, 50, 51], further supports BIRC5 as a viable target for vaccine approaches.

**Figure 5 : BIRC5 gene expression profile across all tumor samples and paired normal tissues; *RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects [35]***

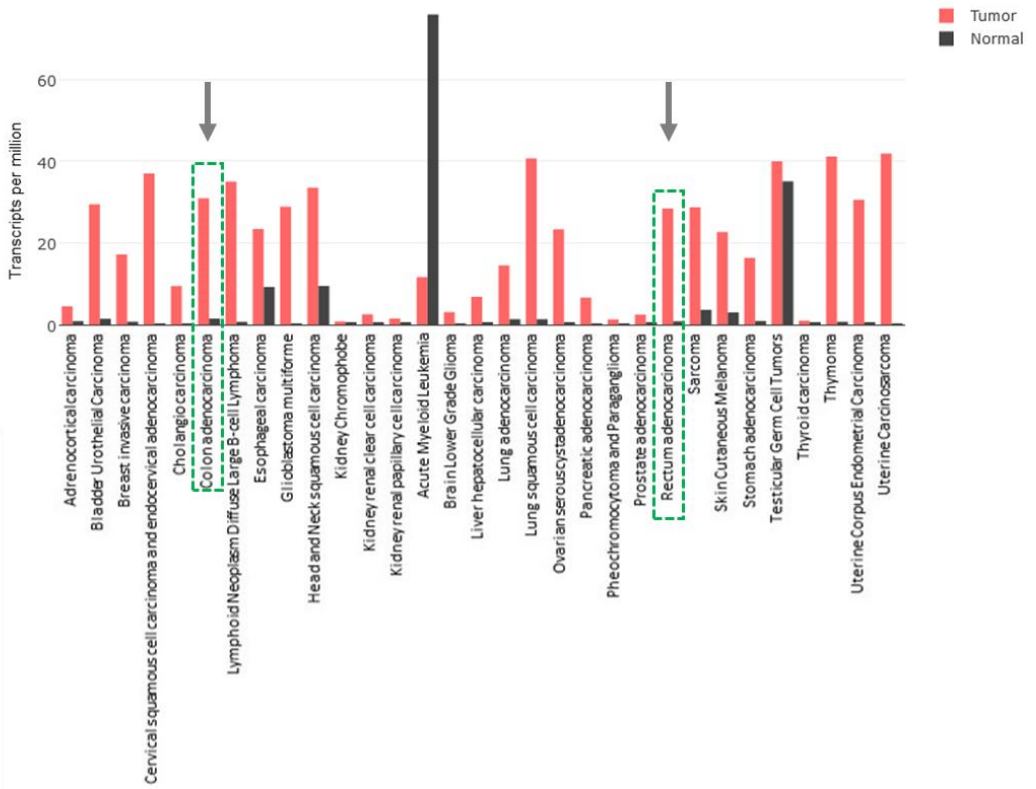
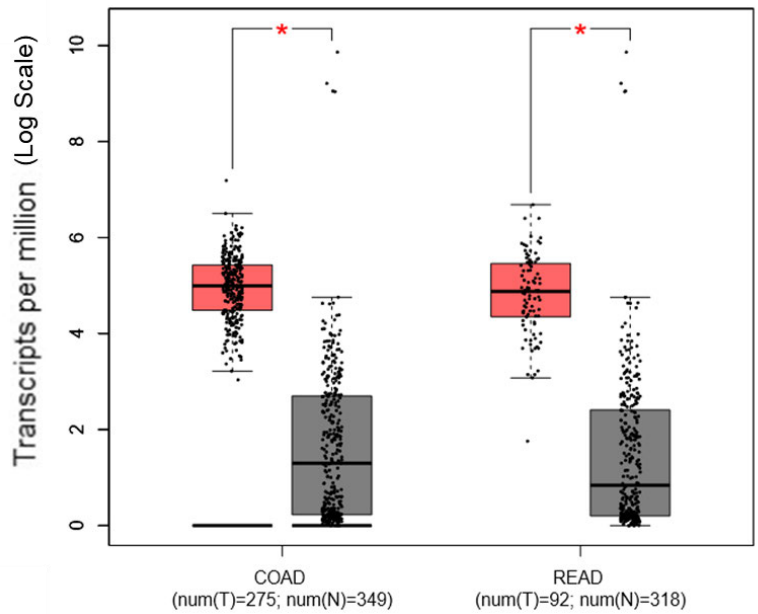



**Figure 6 : BIRC5 gene expression profile across all colorectal samples and paired normal tissues; RNA sequencing expression data of BIRC5 in colon adenocarcinoma (COAD), rectum adenocarcinoma tumors (READ) and related normal samples from the TCGA and the GTEx projects [35]**



In conclusion, BIRC5 has a very high expression in cancer cells as compared to the very low or absent expression in normal tissues (except the immune privileged testis and some lymphocytes), and thus BIRC5 is considered as an attractive anticancer target (see **Figure 5** and **Figure 6**).

Based on overexpression in tumoral tissues and low/absent expression in normal tissues, BIRC5 was considered since a long time as an ideal target for vaccine or therapeutic approaches. Several strategies have been developed to target BIRC5 and have been tested in many clinical trials in many cancer types. This includes cell-based strategies such as BIRC5 pulsed dendritic cells, and BIRC5 peptide vaccines. The safety information regarding the clinical trials targeting BIRC5 is summarised in the current IB of EO2040; in conclusion, there were no treatment limiting adverse events associated with the targeting of BIRC5.

For clinical experience regarding the specific microbiome-derived peptide EO2317, see protocol Section 1.3.2.

### *1.3.2 EO2318 and FOXM1*

EO2318 is a [redacted] peptide identified from the human gut microbiome that targets a cognate T-cell epitope on FOXM1. [redacted]

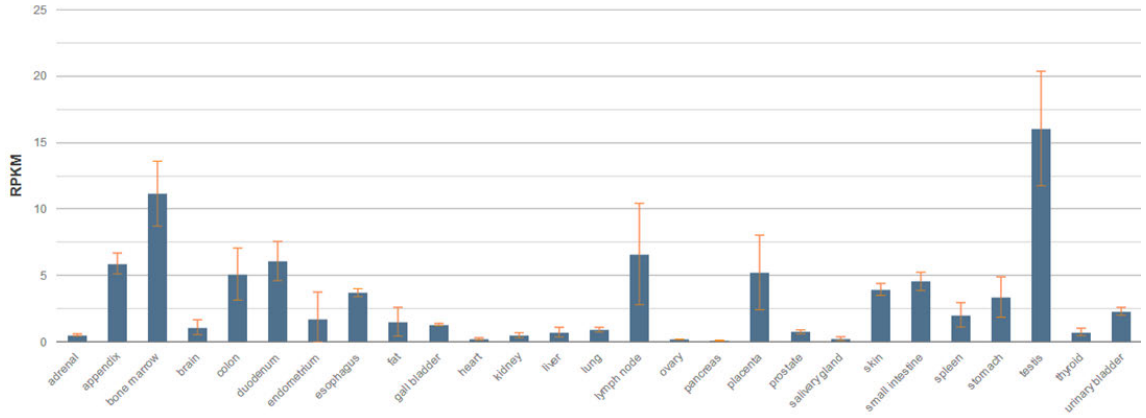
FOXM1, a member of the Fox transcription factors, regulates transcription of cell cycle genes essential for G1-S and G2-M progression, chromosome stability and segregation (including Nek2, KIF20A, CENP-A, AURKB, BIRC5, CENP-F, CDC25A, CDC25B, p27Kip1, cyclin B, and cyclin D1). FOXM1 is also involved in regulation of PI3K-AKT, TGF-β, Wnt/β-catenin, insulin, Sonic-Hedgehog, and Jagged-Notch signalling pathways. A current hypothesis is that abnormal activation of several kinases (such as MST1/2, RAF, RAS or MAPK2) leads to nuclear accumulation of FOXM1 in tumors. FOXM1 has been extensively studied and was shown to drive most of the hallmarks of malignancy. FOXM1 exhibits a proliferation-specific expression pattern, and its expression is regulated by proliferation and anti-proliferation signals as well as by proto-oncoproteins and tumor suppressors [52]. FOXM1 is basically considered to be involved in all stages of oncogenesis [53, 54].

***FOXM1 Expression in normal human tissues:***

FOXM1 is widely expressed in embryonic tissues and shows a low level of expression in normal adult tissues [55, 56]. Analysis of gene expression database shows remaining high expression of FOXM1 in the testis and low expression in hematopoietic cells, esophagus, colon, and skin [57, 58].

The protein expression addressed by massive peptide sequencing does not allow to detect FOXM1 specific peptides in any tissues [32]. Other large-scale data of protein expression highlight the preferential expression of FOXM1 in the testis and hematopoietic cells.

**Figure 7 : Relative FOXM1 mRNA expression in normal tissues; (HPA RNA-seq normal tissues [59]; RPKM: Reads Per Kilobase Million) RNA-seq performed on tissue samples from 95 human individuals representing 27 different tissues**




***FOXM1 Overexpression in colorectal tumor:***

While FOXM1 expression is turned off in terminally differentiated cells, it is upregulated in a multitude of human solid tumors including breast cancer, non-small cell lung carcinoma, hepatocellular carcinoma, pancreatic carcinoma, colon cancer, ovarian cancer, prostate cancer glioblastoma and others [60]. Gene expression profiles across tumor samples and paired normal tissues (from the TCGA and the GTEx projects), confirm the clear overexpression of FOXM1 in many different tumors including colorectal cancer [61]. This overexpression of FOXM1 was proposed to be a major predictor of adverse outcomes across 39 human malignancies by computational analysis [62]. FOXM1 overexpression in colorectal cancer has been documented in many reports both at the mRNA and protein level, with overexpression observed in between 50 % to 85% of analyzed samples [63, 64, 65]. FOXM1 expression is directly linked to prognosis in colorectal carcinoma and FOXM1 levels correlate with cancer progression, lymph node and liver metastasis, and high TNM stages [64, 66,67]. The important role of FOXM1 in metastasis development in CRC is thought to be linked to induction of the epithelial-mesenchymal transition [68, 69].

**Figure 8 : FOXM1 gene expression profile across all tumor samples and paired normal tissues; *RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects [61]***

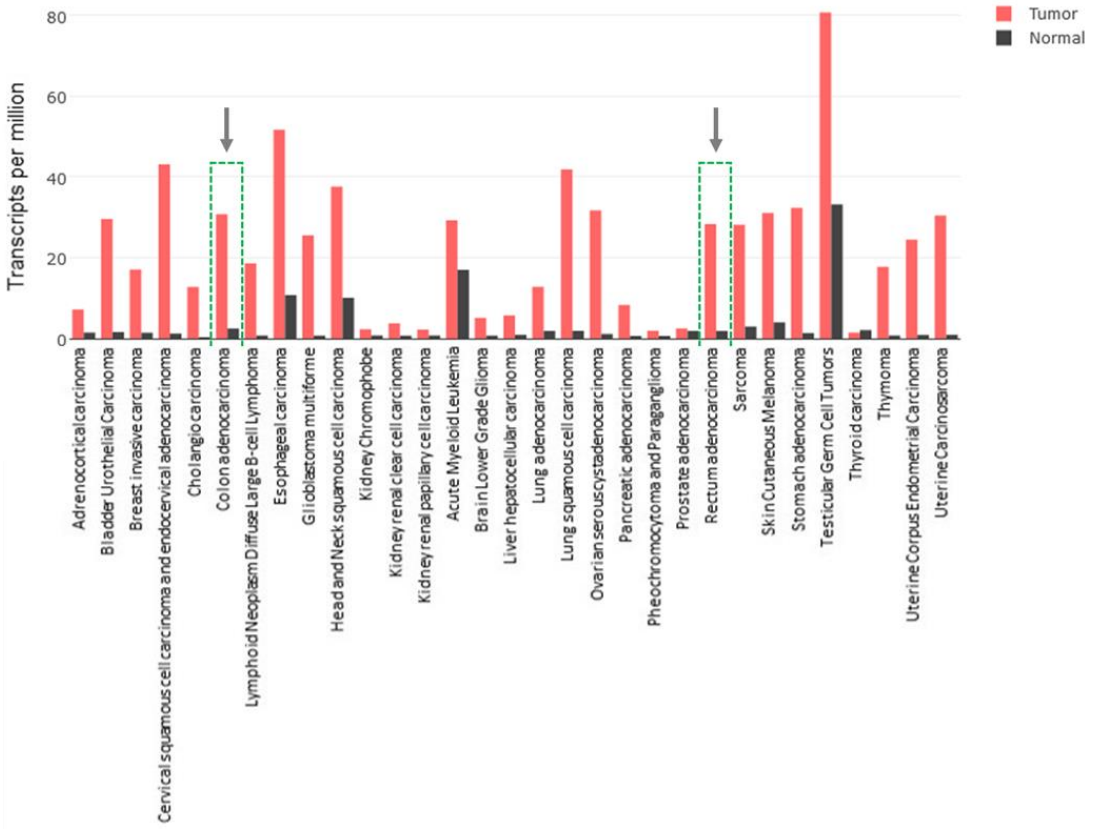
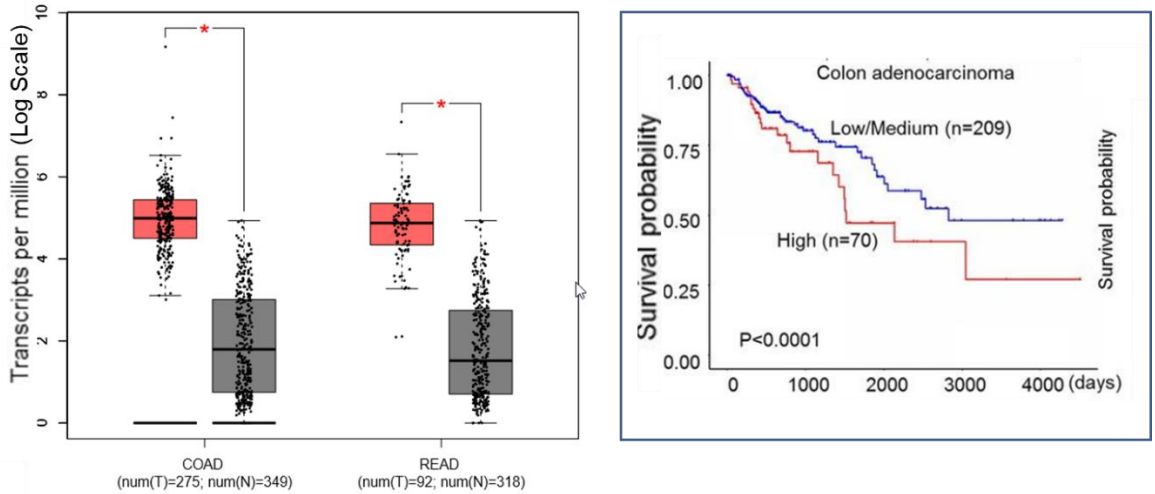



**Figure 9 : FOXM1 gene expression profile across colorectal samples and paired normal tissues, association with overall survival in patients with colon adenocarcinoma; Kaplan–Meier analyses of overall survival according to FOXM1 expression levels in adenocarcinoma patients 70]**



In conclusion, FOXM1 has a very high expression in cancer cells as compared to the very low or absent expression in normal tissues (except the immune privileged testis), and thus FOXM1 is considered as an attractive anticancer target.

Despite the increased interest of FOXM1 as a target of antitumoral therapy [71, 72], to date no specific inhibitors are available for clinical studies, due to the poor druggability of this transcription factor.

Interestingly, several FOXM1 peptides have been described for their ability to prime HLA-A2 or HLA-A24 restricted cytotoxic T lymphocytes in mice [73], suggesting that FOXM1 may be a suitable target for immunotherapy against cancers. Several phase I/II clinical trials were undertaken using FOXM1 peptides, for patients with HLA-A24 with gastric cancer, ovarian cancer, cervical cancer, or glioma. The safety information regarding the clinical trials targeting FOXM1 is summarised in the current IB of EO2040.

For clinical experience regarding the specific microbiome-derived peptide EO2318, see protocol **Section 1.3.2**.

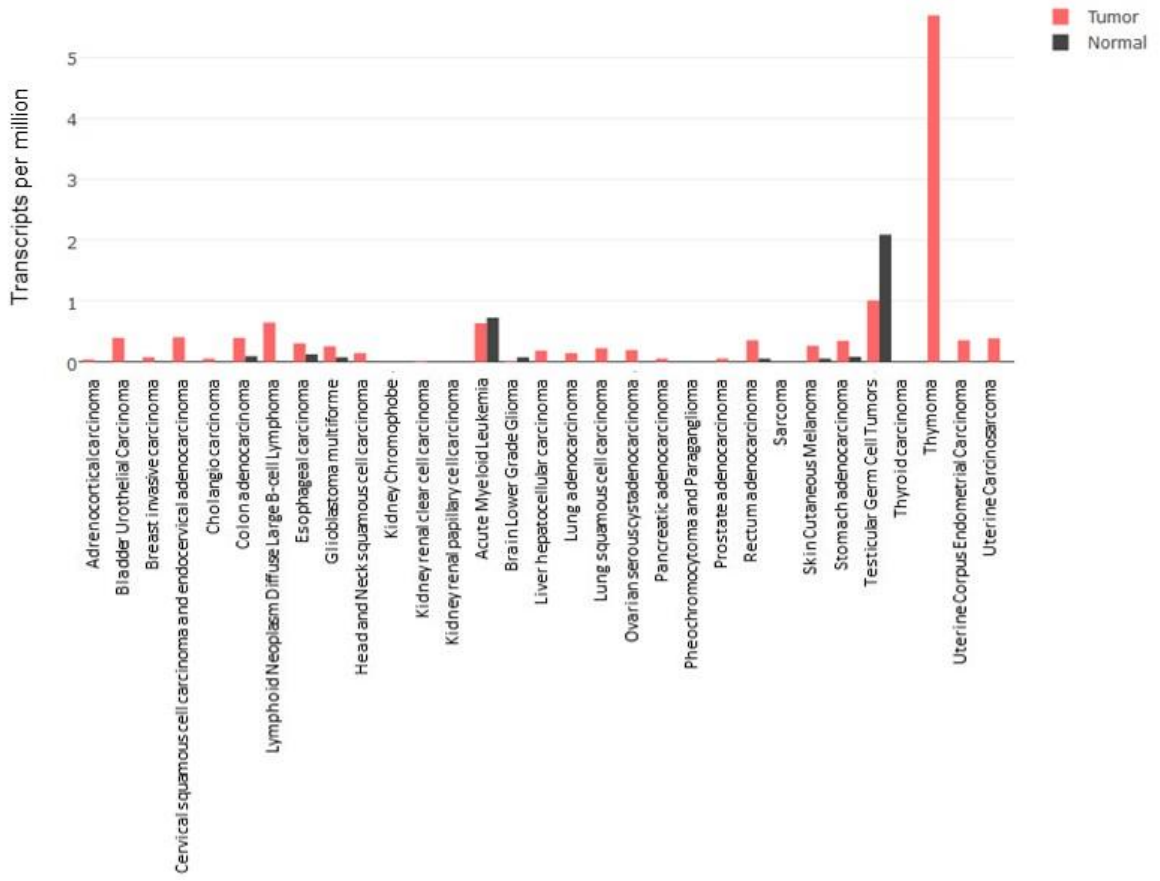
### *1.3.3 UCP2 – Helper peptide*

To generate an efficient immune response, the helper peptide UCP2 is included in EO2040. UCP2 was described by Godet [74]. It is a telomerase reverse transcriptase (TERT) derived CD4 epitope that binds to the most commonly found MHC class II alleles. This Th1 helper peptide should be able to expand T cells with the ability to sustain efficient dendritic cell activation and specific CTL activation by secreting interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α) and interleukine-2 (IL-2) and enhancing expression of costimulatory signals on dendritic cells and T cells [75].

TERT expression is considered to be absent or extremely low in normal tissues (except in testis and some hematopoietic cells) while overexpressed in a large majority of tumors. The role of TERT in tumor progression is well documented. Cancer cells overcome senescence via telomere length maintenance mechanisms involving telomerase activation [76]. TERT overexpression achieved via multiple genetic and epigenetic mechanisms could be observed in 80–90% of malignant tumors [77, 78].

The use of a helper antigen targeting TERT in colorectal cancer is further supported by the fact that telomerase activity and TERT expression were strongly enhanced in 80% to 100% of sample from adenocarcinoma tumors [79, 80, 81, 82], with the potential to be used as a biomarker and prognostic tool [83, 84].

**Figure 10 : TERT gene expression profile across all tumor samples and paired normal tissues; RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects [85]**




The ability of UCP2 to elicit a specific CD4+ T cell responses has been demonstrated in *in vivo* models. Furthermore, spontaneous T cell responses against UCP2 were observed in various types of cancers [86], and this peptide is currently under clinical evaluation in phase 1/2 studies in non-small cell lung cancer and in glioblastoma.

Based on overexpression in tumoral tissues, low/absent expression in normal tissues and a major function in tumor development, TERT was considered since a long time as an ideal target for vaccine or therapeutic approaches.

Several strategies have been developed to target TERT and have been tested in many clinical trials in many cancer types. This includes cell-based strategies such as TERT pulsed dendritic cells, TERT peptide vaccines, and adoptive transfer of T-cells. The safety information regarding the clinical trials targeting UCP2 is summarised in the current IB of EO2040.

In the EO2040 peptide mix, UCP2 is mainly used as a target for helper T cells, to create T helper support for a cytotoxic response against the other targeted antigens and is not used as a target for cytotoxic cells. For clinical experience regarding the use of UCP2 in association with microbiome-derived peptides, see protocol Section 1.3.2.

[redacted]

### *1.3.5 Combination with anti-PD1 blockade*

The concomitant administration of EO2040 and an anti-PD1 blocking agent is utilized to optimize the possibility for tumor specific T cells to infiltrate and effectively function within the tumor environment.

During the initial development of EO2401 for the treatment of solid tumors, nivolumab has been used in combination with EO2401 as the combination of a cancer vaccine and checkpoint inhibitors may function synergistically to induce more efficient anti-tumor immune responses [95, 96].

The mechanism of action of anti-PD1 like nivolumab, blocking interaction between PD-1 and its ligands, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response aims at facilitating access to the tumor by the T cells reactivated and expanded by the microbiome-derived therapeutic vaccine. The final objective being to induce a fast and extensive expansion and infiltration of tumor by the TAA specific T-cells.

Except the strong general rationale combining anti-PD1 blockade with a vaccination approach [97, 98, 99], the rationale for the approach in the current study is also supported by the outcome of a mouse xenograft glioblastoma model utilizing a microbiome-derived peptide and anti-PD1 blockade (**Figure 11**).

### *1.3.6 Early clinical development of microbiome-derived peptides in solid tumors*

EO2040, the compound to be used in the current trial is a derivative of the compound EO2401. EO2040 include the same components as EO2401, except the microbiome derived peptide EO2316, mimicking an epitope on the TAA interleukin 13 receptor alpha-2 (IL-13Rα2). The reason to exclude peptide EO2316 from compound EO2040 is that IL-13Rα2 currently does not have a rationale to be used when colorectal cancer is targeted, i.e. expression levels as known today are not high enough.

In the following, the parent compound of EO2040, i.e. EO2401, is described regarding early clinical development, especially with regard to safety, since it is assumed that the safety profile of EO2040 will be at pair with, or better than for EO2401. Note, per end-January 2022, more than 100 patients with glioblastoma and adrenal tumors had started treatment with EO2401 in combination with nivolumab showing the ability to generate strong systemic immune responses against the targeted TAAs and being well tolerated; the safety profile being consistent with the profile of the combination partner nivolumab, with the only addition of local administration site reactions (most commonly grade 1, erythema, induration, and sometimes pain).

The initial clinical development of EO2401 include two phase 1/2 trials, one in glioblastoma (EOGBM1-18; EudraCT# 2018-002279-16, IND# 19229) and one in adrenal tumors (EOADR1-19; EudraCT# 2019-003396-19, IND# 19229).

The early clinical development of EO2401 aimed at showing that therapeutic cancer vaccination with EO2401 in combination with anti-Programmed cell Death protein 1(PD1)

blockade is safe and tolerable, and can achieve expansion of T cells not only recognizing the microbiome derived peptides used for immunization but also recognizing the targeted nominal TAAs expressed by human tumor cells in patients with malignancies (i.e. immunological proof of concept). In addition, preliminary efficacy aspects of EO2401 as well as multiple translational exploratory endpoints are included in the early development trials.

The first-in-human (FIH) trial of EO2401, EOGBM1-18, is a multicenter (10 sites in France, Germany, Spain, and USA), phase 1/2 trial, in patients with unequivocal evidence of progressive or first recurrent glioblastoma. The study design builds on an initial cohort for assessment of mainly safety and tolerability of monotherapy EO2401, and EO2401 in combination with nivolumab (a cohort with a 3-by-3 design), and expansion cohorts for assessment of further safety and tolerability, immunogenicity, and preliminary efficacy during treatment with EO2401 in combination with nivolumab (without any EO2401 monotherapy component), and EO2401 in combination with both nivolumab and bevacizumab concomitantly [redacted] The study was initially planned to enroll a maximum of 52 patients but has since been expanded to approximately 80 patients, and the first patient treated in trial EOGBM1-18 received EO2401 monotherapy on July 28, 2020. See Section 1.3.6.1 for preliminary outcome-information related to trial EOGBM1-18.

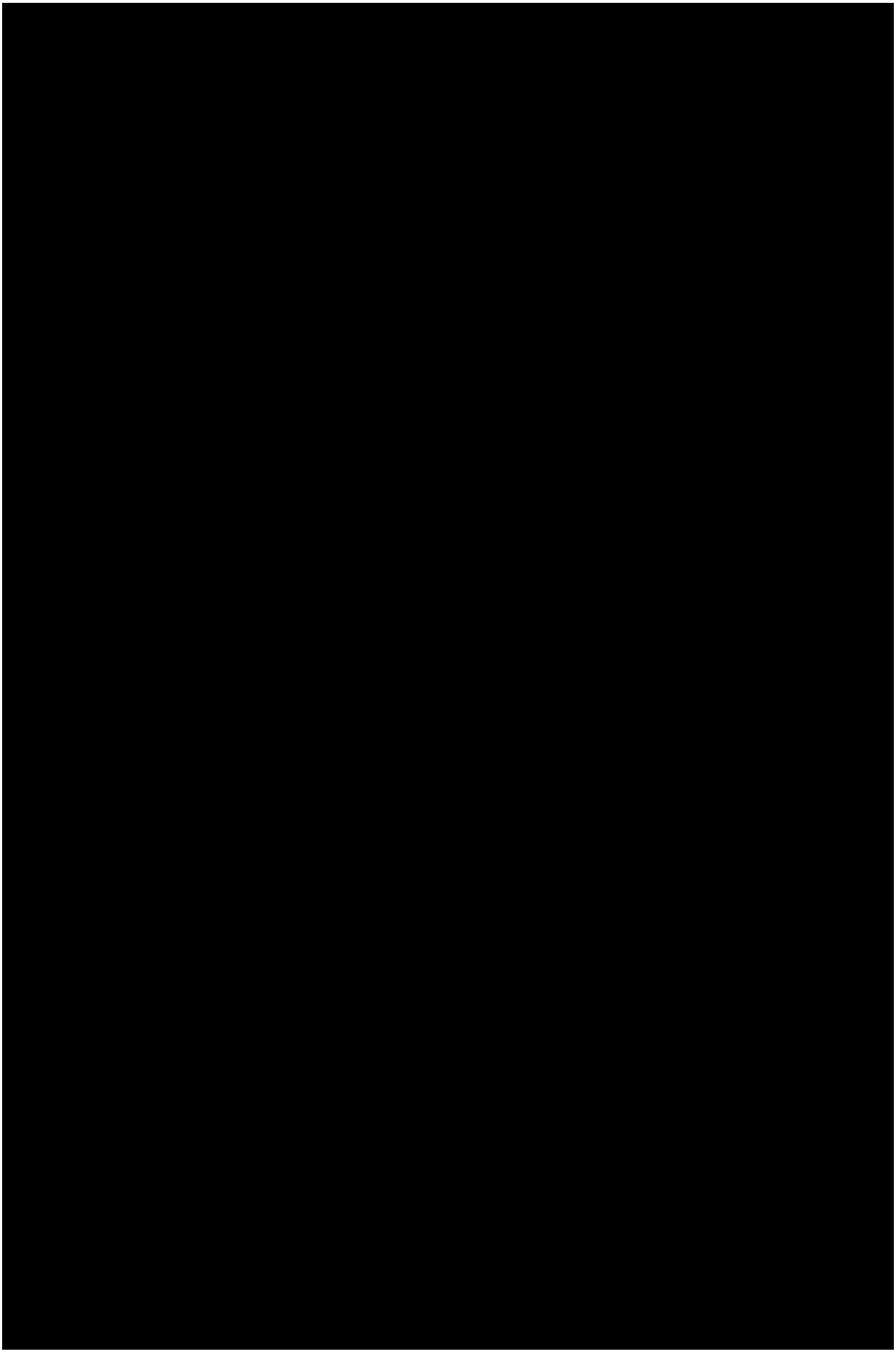
The second early clinical development trial including EO2401, EOADR1-19, is a multicenter (10 sites in Denmark, France, Germany, Italy, Netherlands, Spain, Sweden, and USA), phase 1/2 trial, in patients with adrenocortical carcinoma or malignant pheochromocytoma/paraganglioma. The study design builds on an initial cohort for assessment of safety and tolerability of EO2401 in combination with nivolumab (a cohort with a 3-by-3 design), and expansion cohorts for further assessments of safety and tolerability, immunogenicity, and preliminary efficacy in specific subgroups of patients with adrenal tumors (previously untreated, or previously treated, with each adrenal tumor entity).

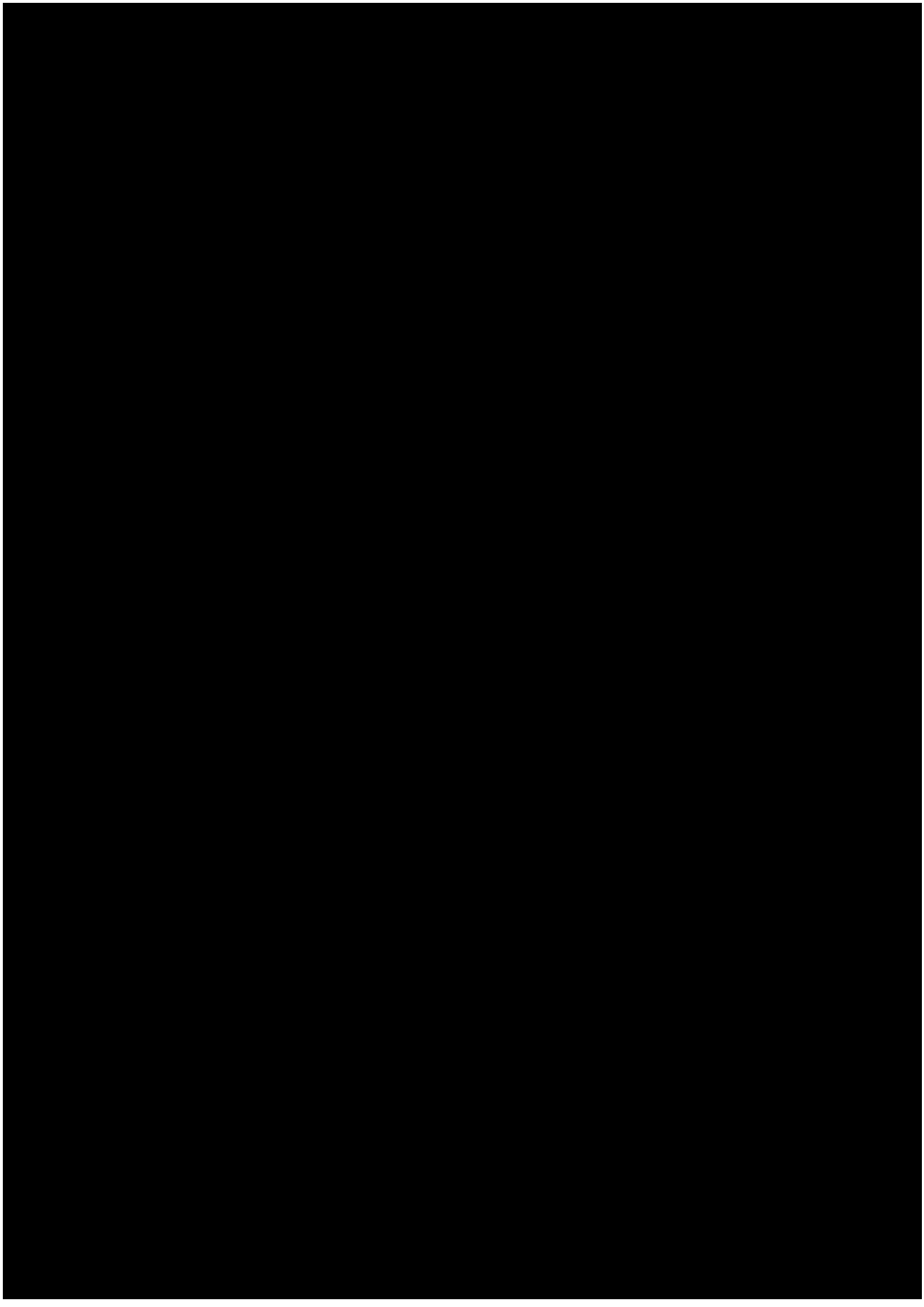
The study was initially planned to enroll 60 patients in the expansion phase cohorts after the initial safety cohort was finalized but is now to be expanded beyond this with a randomized component including 65 patients with previously treated adrenocortical carcinoma. The first patient treated in trial EOADR1-19 received EO2401 in combination with nivolumab on August 11, 2020.

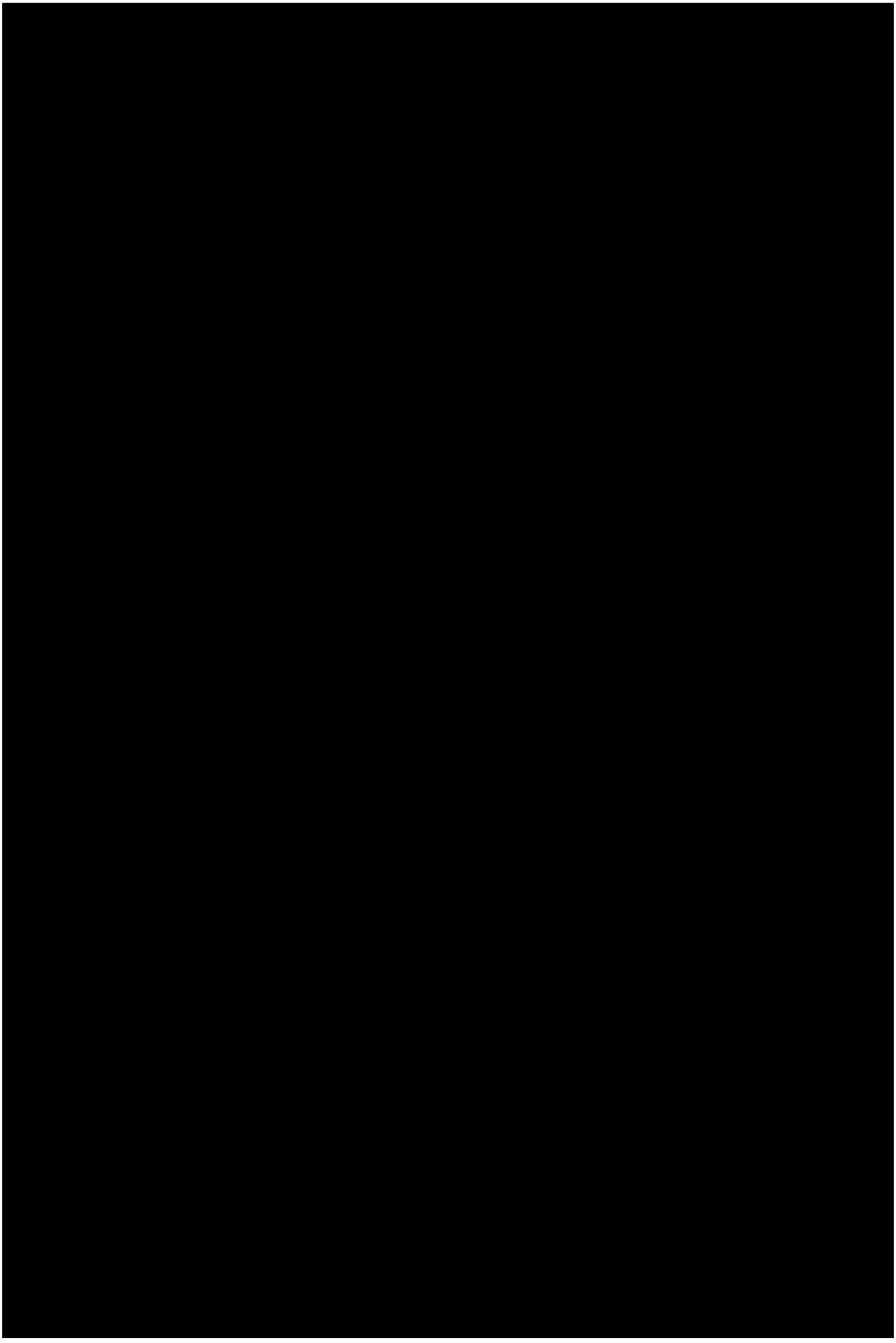
The trials EOGBM1-18 and EOADR1-19 are overseen by independent data monitoring committees (IDMCs) which are cross-sharing information.

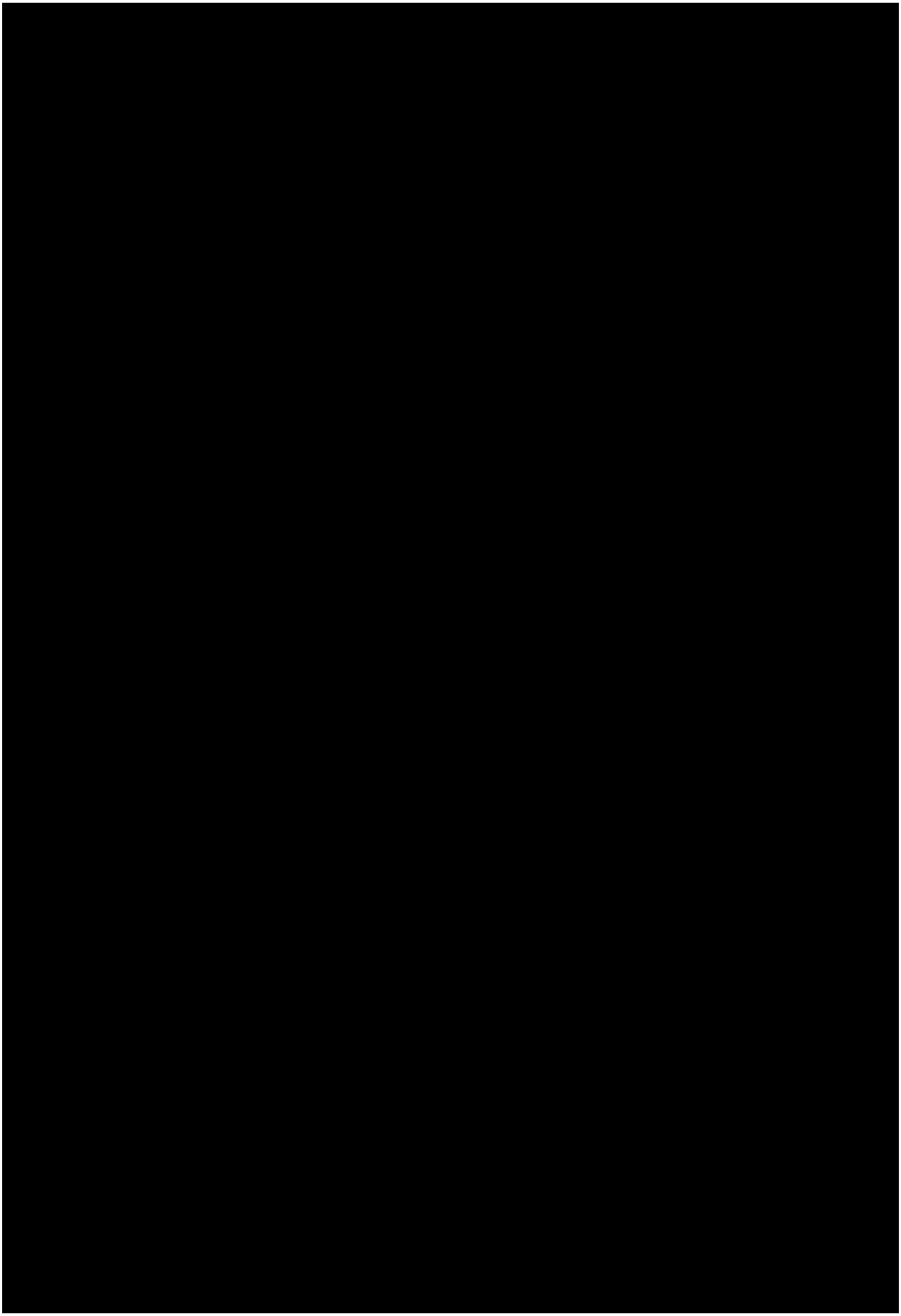
For completeness, it should be mentioned that the microbiome-derived mimicry therapeutic vaccine concept is also applied with a second compound, EO2463, targeting B cell markers, in a clinical trial in follicular and marginal zone lymphoma (EONHL1-20; EudraCT 2020-003999-40). The first patient treated in trial EONHL1-20 received EO2463 monotherapy on July 5, 2021. Even if clear inter-study differences regarding populations/compounds, the IDMC for trial EONHL1-20 is also participating in the cross-sharing of information with the IDMCs for trials EOGBM1-18 and EOADR1-19 to enhance the possibility to detect possible concept related safety signals. No such signal has been found currently.

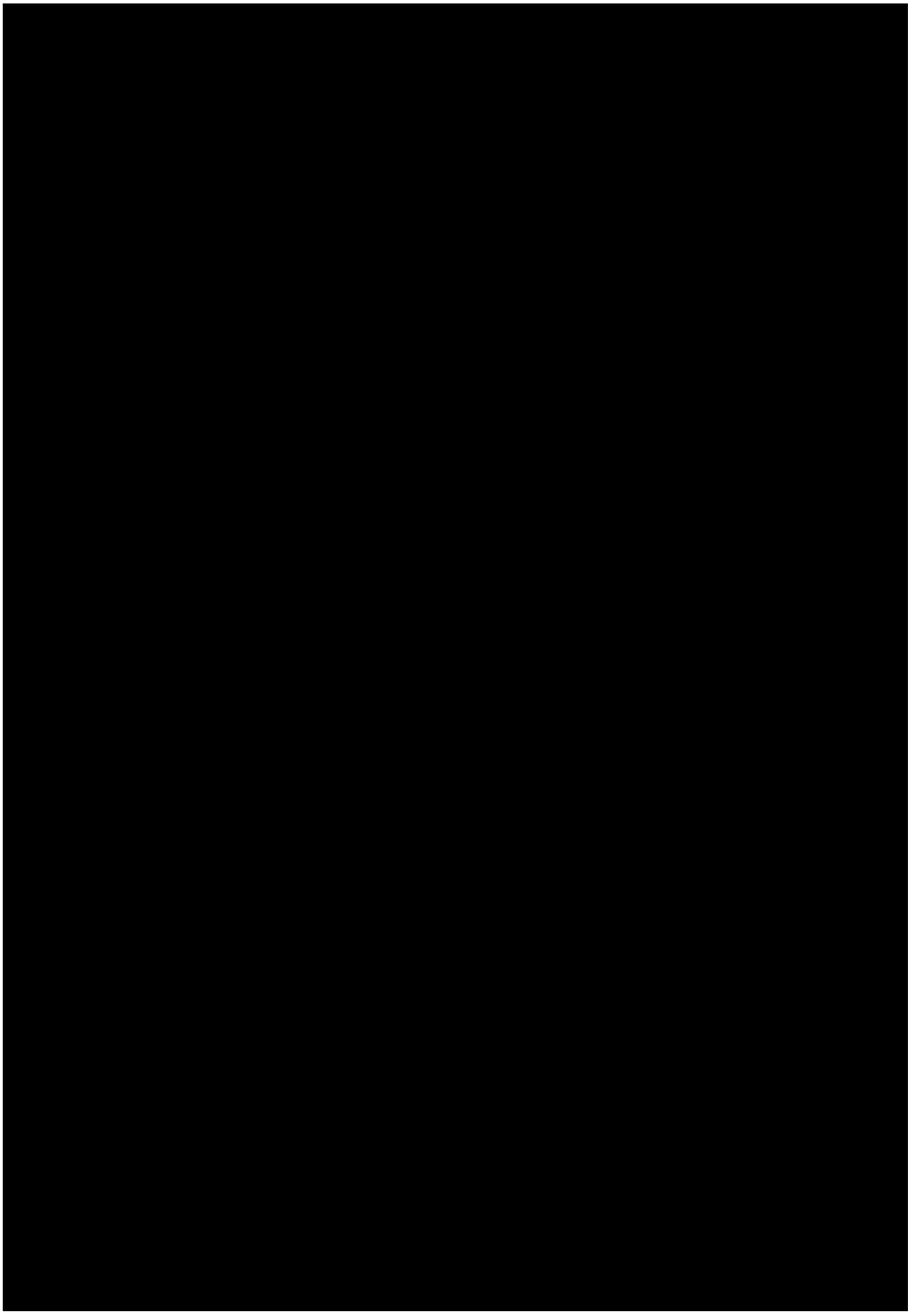
[redacted]

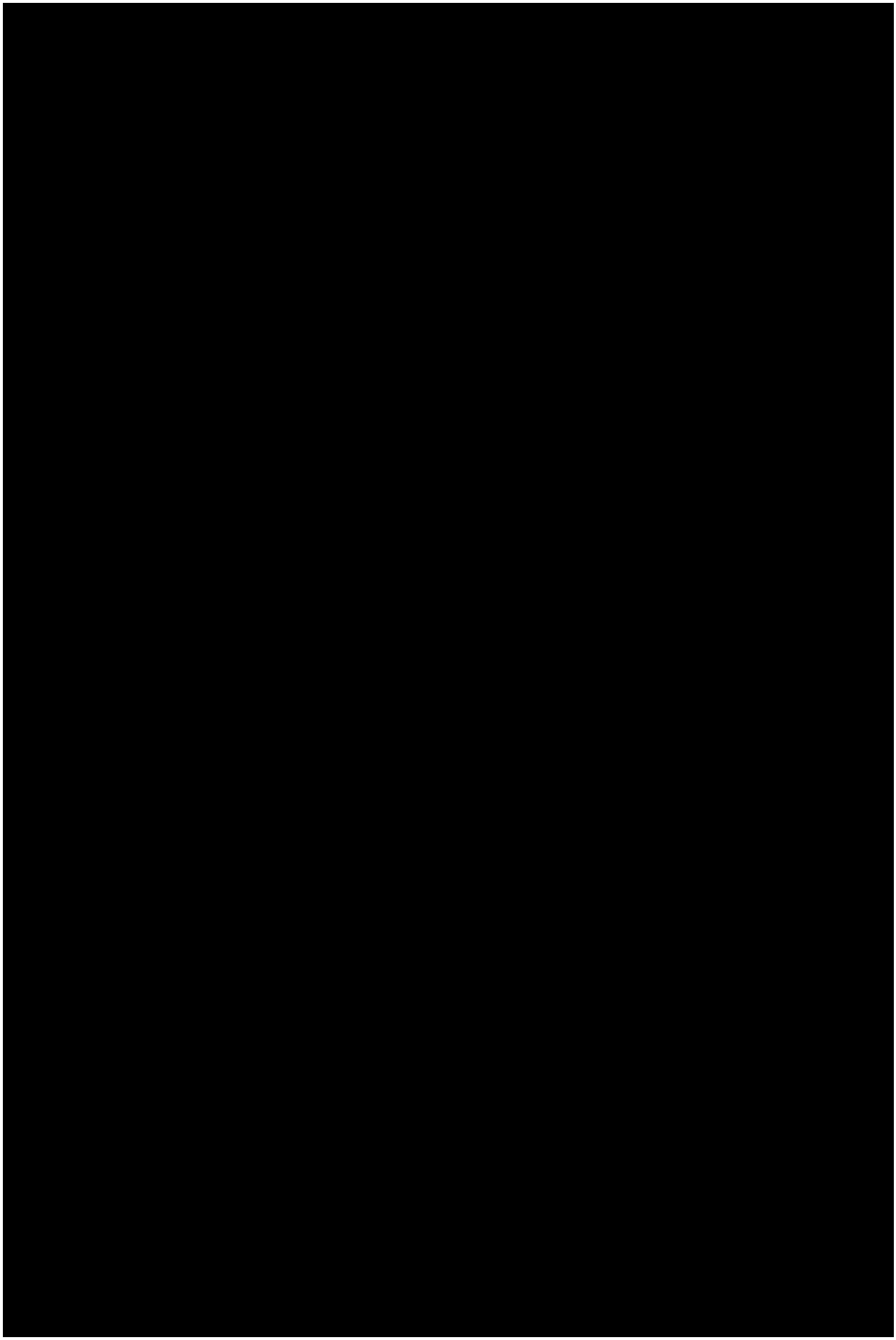


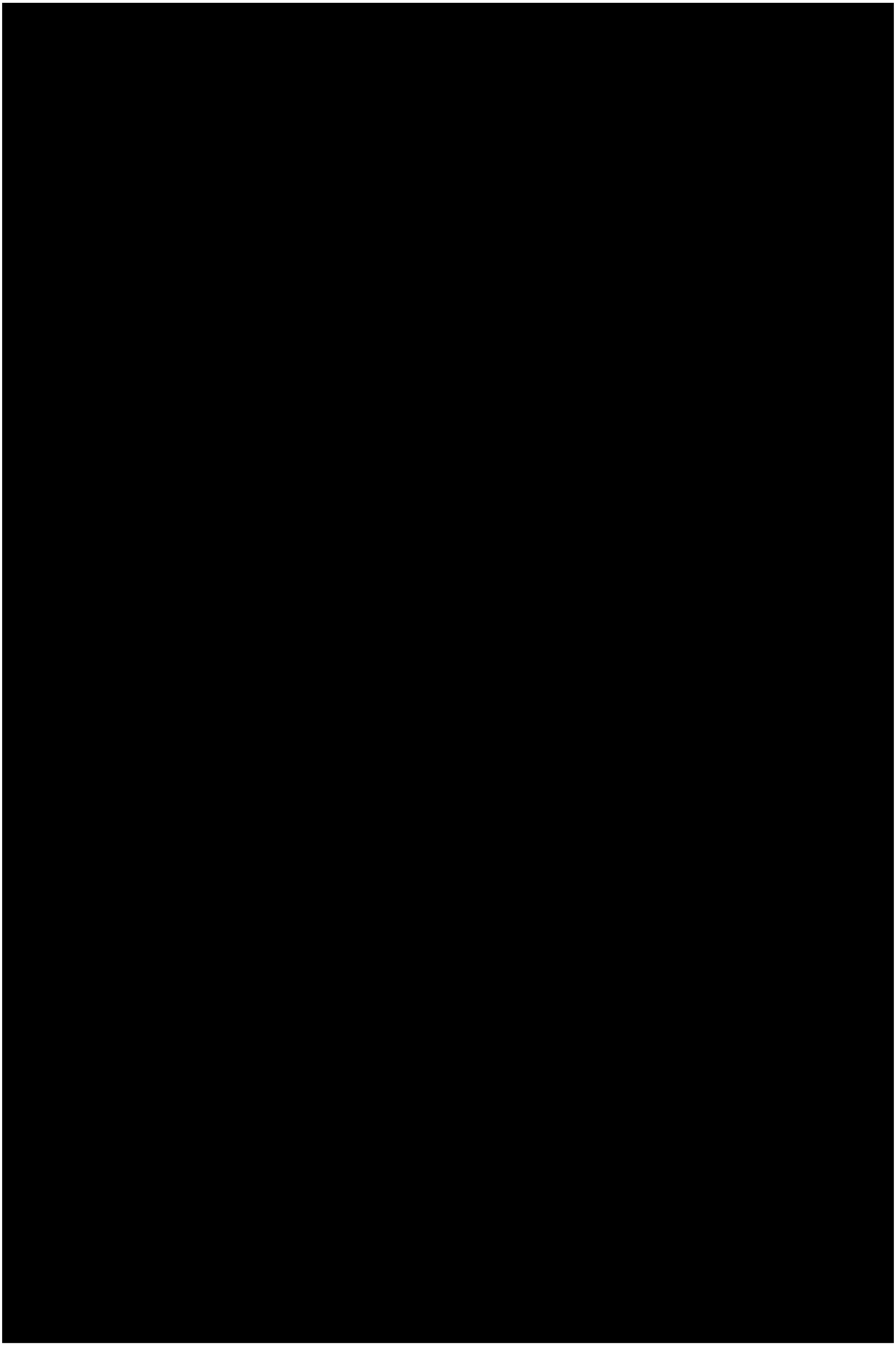


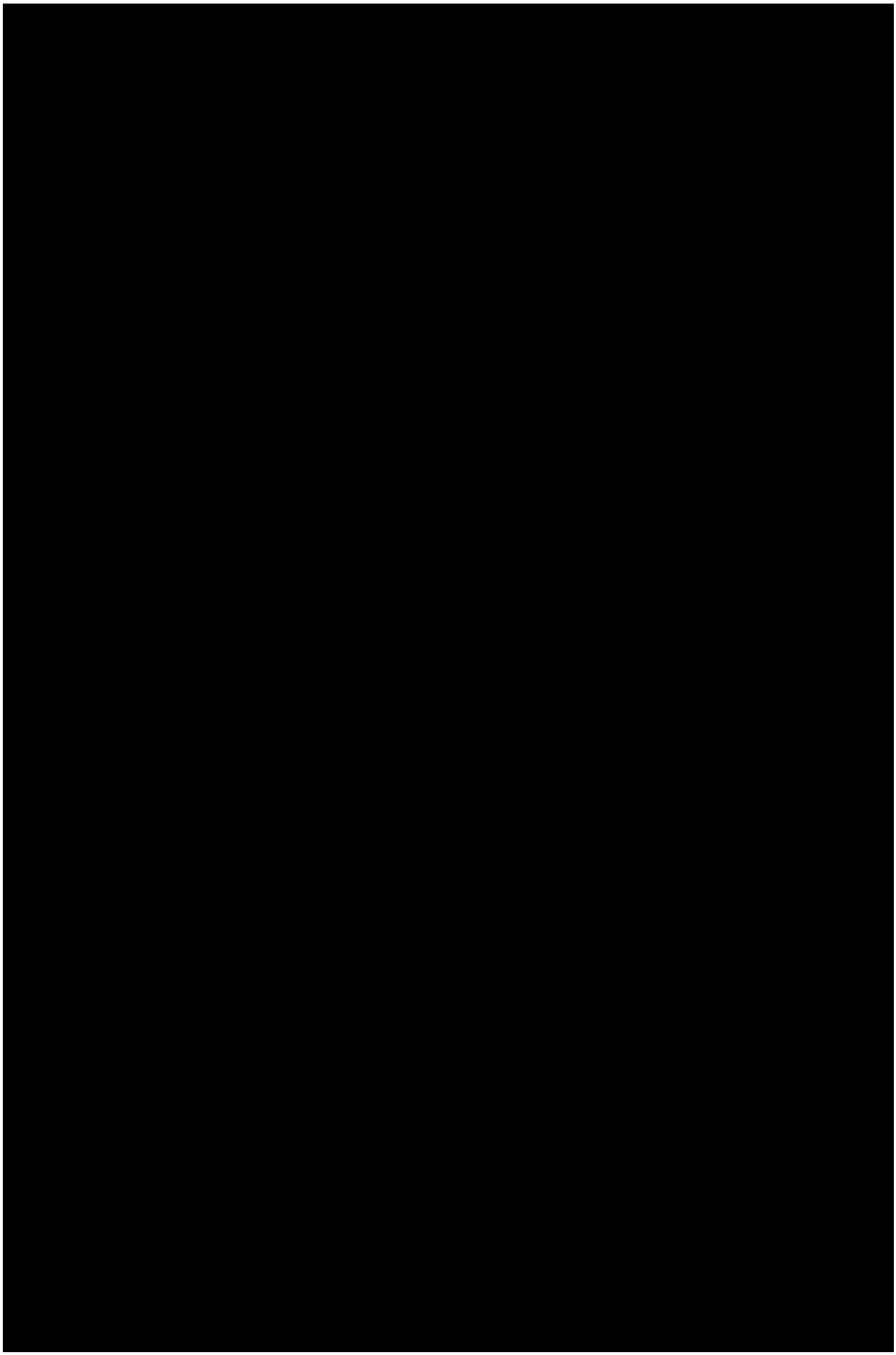












[redacted]

**Overall conclusion regarding safety of EO2401**

Overall, taking into account the specifics of the differences in patient populations, the early development safety profiles for EO2401 in trials EOGBM1-18 and EOADR1-19, seems similar and showing that the compound is well tolerated with a safety profile consistent with the safety profile of nivolumab monotherapy (plus bevacizumab monotherapy when applicable), except the addition of local administration site reactions.

It is currently assumed that the differences seen in some parameters describing local administration site reactions between trial EOGBM1-18 and EOADR1-19 are related to the sometimes-short follow-up and still ongoing treatments.

#### *1.3.6.2 EO2401 clinical efficacy and immunogenicity*

[redacted]

[redacted]

[redacted]

### *1.3.7 Benefit/risk assessment and description of and justification for the route of administration, dosage, dosage regimen, and treatment period*

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 rationale for the proposed study is outlined in Section 2.1, indicating the current lack of therapy options for patients with ctDNA defined MRD after completion of all available standard of care curative treatments, and thus the need for new therapeutic approaches.

Application of rationally developed immunotherapies might be one avenue to explore the possibility to enhance the survival chance for patients with ctDNA defined MRD recurrence/progression of CRC. An innovative option which has been tested in the clinical phase 1/2 setting and shown to have a predictable safety profile and indications for the possibility of efficacy in solid tumors, is the use of microbiome-derived peptide vaccination targeting tumor associated antigens (TAAs) highly expressed, and linked to clinical outcome, in CRC, in combination with anti-PD1 blockade in the form of nivolumab.

EO2040, the compound to be used in the current trial is a derivative of the compound EO2401. EO2040 include the same components as EO2401, except the microbiome derived peptide EO2316, mimicking an epitope on the TAA interleukin 13 receptor alpha-2 (IL-13Rα2). The reason to exclude peptide EO2316 from compound EO2040 is that IL-13Rα2 currently does not have a rationale to be used when colorectal cancer is targeted, i.e. expression levels as known today are not high enough.

The components of EO2040 are two microbial-derived peptides (EO2317 and EO2318) mimicking cytotoxic T cell (CD8+ T cell) HLA-A2 restricted epitopes from two different TAAs, BIRC5/survivin and FOXM1, respectively, and the helper peptide (CD4+ T cell epitope) universal cancer peptide 2 (UCP2), derived from hTERT.

For details regarding the non-clinical safety evaluations of the microbiome derived therapeutic vaccine concept, including EO2040, see the current IB of EO2040, in summary:

- Neither off-target (non-specific) nor other adverse effects that may occur following binding of the peptides to HLA molecules have been observed in HLA-A2 transgenic mice.
- It has been demonstrated in wild type mice that activation of T cells by vaccination with mouse microbial-derived peptides does not cause toxic immune reactions against microbial gut contents; the obtained results suggest good tolerability and no obvious signs of toxicity.
- Assessment of the safety of the concomitant administration of microbial-derived peptides with anti-PD1 showed that the combination of mouse bacterial peptides with anti-PD1 revealed no sign of toxicity, confirming that induction of a systemic immune response against a bacterial antigen does not affect gut homeostasis.

The safety, and benefit/risk, profile of nivolumab is well known via the multiple labels for different malignant diseases already approved globally, also including a subgroup of patients

with CRC (mismatch repair deficient (dMMR) or microsatellite instability-high (MSI-H) metastatic disease after prior treatment) [90, 91].

The parent compound of EO2040, i.e. EO2401, in combination with nivolumab, has been thoroughly investigated in two phase 1/2 trials in more than 100 patients (by end-January 2022) with glioblastoma and adrenal tumors, showing the ability to generate strong systemic immune responses against the targeted TAAs, and being well tolerated; the safety profile being consistent with the profile of the combination partner nivolumab, with the only addition of local administration site reactions (most commonly grade 1, erythema, induration, and sometimes pain). The clinical experience to date for EO2401 [redacted] in combination with nivolumab, and nivolumab/bevacizumab is described in Section 1.3.2.

The overall assessment of the Sponsor is that the safety profile expected for the current trial, as already indicated by the experience in phase 1/2, of the combination of EO2401 with nivolumab, will be like the well described safety profile of nivolumab given without EO2040, with the addition of local administration site reactions (erythema, induration, and sometimes pain) in around half of the patients provided longer treatment durations are achieved. The median duration of local administration site reactions is expected to be as in trial EOGBM1-18, i.e. around 6 weeks, assumed without the need to adjust study treatment delivery due to the applied rotating administration sites per below.

The location of EO2040 injections is rotating, i.e. an injection site will be used every fourth time, meaning that during the current trial with a pre-determined treatment duration of maximum 6 months, each administration site will only be used twice); the injection sites are right and left upper extremity, and right and left lower extremity/inguinal area, with the more specific locations being:

- for upper extremity = area of armpit, or deltoid muscle of arm, depending on the constitution of the patient, and
- for lower extremity = area of groin, or anterolateral thigh muscle, depending on the constitution of the patient.

[redacted]

The selection of antigens targeted by EO2040 and their expression in normal tissues makes it unlikely that other normal cells, would be targeted by EO2040 expanded T cells; for further details see Sections 1.3.1 1.3.2, and 1.3.3, and the current version of the IB for EO2040. However, provided EO2040 induces an efficacious expansion of T cells specific for the targeted TAAs with ability to kill tumor cells expressing one, or both, of these TAAs, it cannot be ruled out that such antigen specific T cells might also recognize these antigens if expressed, and presented by MHC, at a high enough level (i.e. expressed as in tissue from CRC) on non-cancerous cells. Thus, if killing of tumor cells by T cells expanded by EO2040 immunization could occur, normal cells (with the same high level of expression as CRC; i.e. unlikely situation - see above) might also be killed by such T cells leading to symptoms like for the immune-related adverse reactions which might be seen during treatment with anti-PD1 (or similar compounds) alone (e.g. see the European SmPC or the US PI for nivolumab [90, 91]. It can be assumed that the immune-related adverse reactions seen during treatment with e.g. nivolumab are driven by self-reactive T cells, even if in the case of nivolumab the

specific targets for these T cells are not known due to the non-specific mode of action of nivolumab in relation to antigen-specificity. Thus, the mechanism for possible normal tissue toxicity by EO2040 immunization is assumed to be the same as for nivolumab induced immune-related toxicity and by that safety measures have been instituted which directly follows the measures already well established for nivolumab and similar compounds (see Section 6.4.1). Of note, the early clinical development of EO2401 does not indicate that there is an added frequency of immune-mediated adverse reactions by the combination of EO2401 and nivolumab, as opposed to administration of nivolumab monotherapy; see Section 1.3.2 [90, 91].

Generally, anti-tumor therapeutic vaccination approaches tested to date have indicated the need for booster doses to maintain any assumed meaningful level of immunity against the targeted antigens, and even in the context of prophylactic vaccinations against infective agents with assumed very potent none self-antigens it is usually not possible to achieve efficacious long-term immunity without booster doses. These general phenomena linked to vaccination strategies indicate that it should also for EO2040 enhanced immunity against the targeted antigens be a possibility to influence efficacy by stopping booster doses. Thus, a meaningful safety action for the applied therapeutic vaccination strategy is to stop EO2040 administrations at non-acceptable toxicity.

In this trial, as in the early development of EO2401, subcutaneous (SC) administration of EO2040 will be applied based on the general notion that this is a well-accepted, and clinically easy and reproducible way for vaccine administration.

[redacted]

The dosage regimen for EO2401 including a priming schedule was chosen to induce an efficient immune response (every 2 weeks, 4 times), and to avoid exhaustion by a 4-week break thereafter. The priming period is followed by a boosting period with injections every 4 weeks to maintain an adequate immune response. The prime-boost approach was based on general immunization concepts, especially from the field of therapeutic cancer vaccines.

The clinical data generated for EO2401 supports the dose and schedule which is implemented in the current trial for EO2040 (see Section 1.3.6.1).

Nivolumab route of administration, dosage, dosage regimen, and treatment periods are aligned with current labeling for the compound [90, 91]. Of note, the interval between nivolumab administrations in the current trial is adjusted according to the administrations of

EO2040, i.e. with every 2 weeks administrations 4 times followed by every 4 weeks during the boosting period of EO2040 when given from the start of study treatment. The nivolumab schedule of administration was selected based on the wish to minimize the number of necessary patient visits.

The optimal duration of immunotherapy in general is an important question without any current definitive answer. Likewise, and even to a greater extent in the situation of ctDNA defined MRD in patients with curatively treated CRC, there is no obvious guidance from prior trials which treatment duration would be optimal. From an immunogenicity standpoint data generated in the development of EO2401 indicate that a very fast expansion, after 1-2 administrations, of T cells with killing ability of cells expressing the targeted TAAs is to be expected for EO2040; [redacted]

Thus, it is known that a fast and prolonged immune response against the targeted TAAs can be achieved with the components of EO2040. However, since it is not known if this immune response can irradicate ctDNA defined MRD in patients with CRC, it seems reasonable to define a finite treatment duration for a first exploratory trial. The selected treatment duration is 6 months, based on the ease of timing with standard of care procedures as further ctDNA testing and radiographic imagining.

Stopping study treatment early at confirmed progression, or individual patient safety concerns is considered standard oncology practice.

The safety profile of the components included in EO2040 [redacted] in combination with nivolumab shown in the early clinical development of EO2401, was as expected based on the components of treatment, and considered supportive for a limited size phase 2 trial in patients with stage II-IV colorectal cancer with ctDNA defined MRD after completion of curative therapy.

In addition, the early development of EO2401 indicated the potential of efficacy of the included vaccine components in combination with nivolumab, in established advanced solid tumors as glioblastoma and adrenocortical carcinoma (the latter in a population of patients where the majority have liver metastases, most often combined with other metastatic sites, including lung).

Based on all above, the Sponsor assessment is that there is a positive benefit/risk ratio for performing the current trial as outlined in protocol EOCRC1-22.

## 1.4 History of amendments

| EOCRC1-22 protocol version (date) | Key points of protocol version (only substantial changes listed) |
|---|---|
| Version 1.0 (2022-MAR-28) | For authority submissions |
| Version 2.0 (2002-JUL-04) | Updated version following IND review by the FDA |

# 2 RATIONALE

## 2.1 Rationale for the study

Detectable ctDNA after completion of curative therapies can identify patients with high-risk CRC, but still with a very limited tumor burden. Thus, a minimal residual disease (MRD) setting which can be assumed to be advantageous, especially for some treatment options, e.g. immunotherapy approaches. The MRD setting has, since a longer time, been targeted for curative treatment approaches with some success in hematological malignancies.

Patients with ctDNA defined MRD of CRC after completion of curative therapies are highly likely to recur with overt metastatic disease (positive predictive value of ctDNA is approximately 100% by 3 years).

Patients with ctDNA positive testing have disease shedding tumor DNA into the circulation, which at early stages might represent radiographically undetectable micrometastatic disease.

The lead-time of ctDNA positivity to radiographic recurrence is up to 6-9 months that provides a window for early initiation of systemic therapy towards eradication of micrometastatic disease [22]. Clearance of ctDNA may be used as a surrogate endpoint under the assumption that this is necessary (albeit insufficient as of now) for eventual cure [93, 94].

We hypothesize that EO2040 in combination with nivolumab in patients with ctDNA-defined MRD will eradicate micrometastatic disease resulting in ctDNA clearance and improvement in disease free survival (DFS) compared to MRD-positive historical controls. The background for this hypothesis has been outlined in Section 1.3.

Patients with CRC being treated with curative intent, dependent on institution and country of treatment, might routinely have ctDNA analysis at key time points including at diagnosis, completion of each line of therapy, post-surgery, post-adjuvant therapy, and during surveillance. Screening for ctDNA-defined MRD is assumed to be done as standard-of-care per current practices.

# 3 STUDY OBJECTIVES AND ENDPOINTS

## 3.1 Objectives

### *3.1.1 Primary objective*

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

*and in sequence, provided the first objective has a positive outcome per 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 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 **Section 7.8**).*

### *3.1.2 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:

1. safety and tolerability of study treatments,
2. the 3-month ctDNA clearance rate,
3. progression of colorectal cancer and death as disease-free survival (DFS),
4. overall survival (OS),
5. survival at 36 months after start of study therapy, and
6. induction/expansion of T cells specific for EO2040, the components of EO2040, and the targeted nominal TAAs (BIRC5 and FOXM1).

### *3.1.3 Exploratory objectives*

1. [redacted]

[redacted]

3. 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.
4. [redacted]

## 3.2 Endpoints

### *3.2.1 Primary endpoint*

Response to therapy will be measured with a standard-of-care, CLIA certified ctDNA assay, or similar certification outside of the USA, at the evaluation times specified in Section 7.1.

Identification of somatic mutations in the blood will be confirmed by sequencing of the resected tumor using a CLIA-certified next-generation sequencing panel. 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.

The primary endpoint is response to treatment at 6 months (see 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 Section 7.8 for rules of assessment of an individual patient best overall response).

[redacted]

### *3.2.2 Secondary endpoints*

1. 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 [100]. See further Section 7.7.
2. 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 Section 3.2.1).

3. Disease-free survival (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.
4. Overall survival (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.
5. Survival at 36 months after start of study treatment, estimated via Kaplan-Meier estimates.
6. 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]

### *3.2.3 Exploratory endpoints*

[redacted]

[redacted]

3. 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]

[redacted]

# 4 STUDY DESIGN

## 4.1 Overall trial design and timelines

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 objectives of the trial are outlined in Section 3.1.

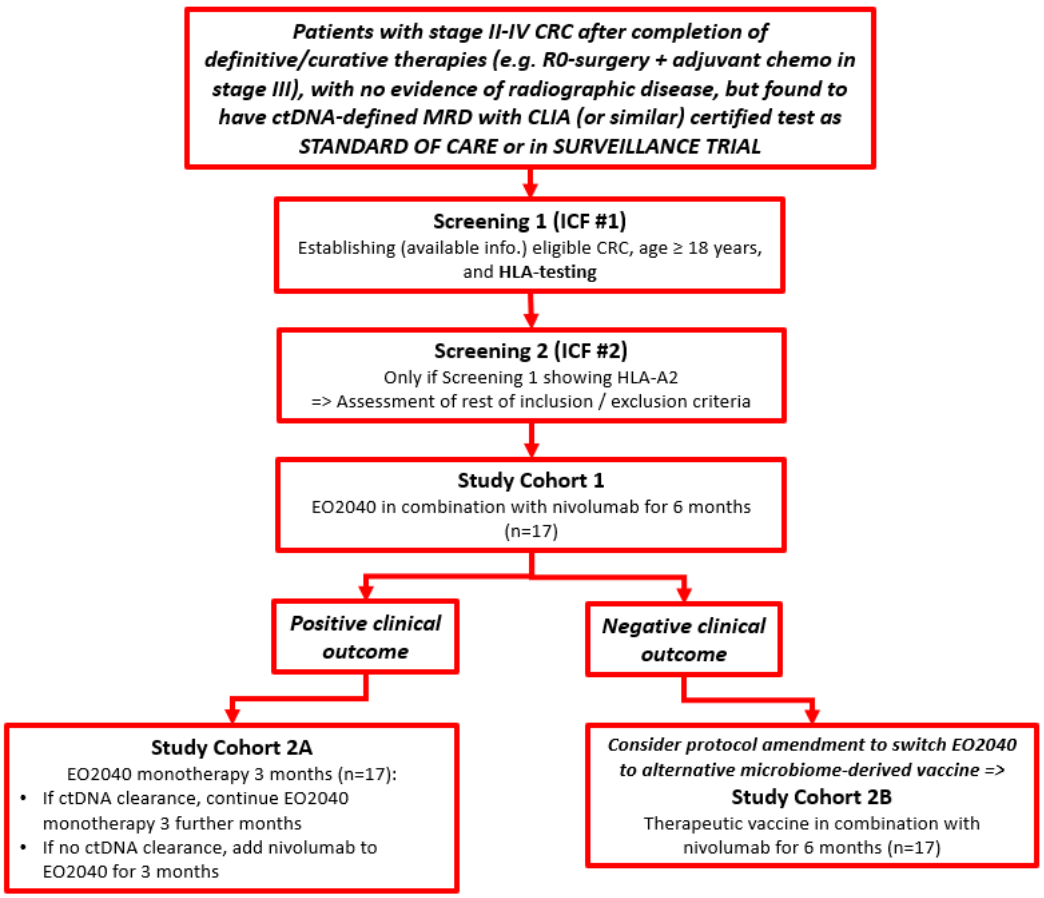
The study is assumed to recruit a total of 17 patients in Cohort 1, and if cohort 1 has a positive outcome (see Section 9), a total of 17 patients will also be recruited to Cohort 2A.

[redacted]

For details regarding sample size determination and statistical analyses see Section 9.

**Figure 15** gives a schematic overview of the trial design, and an overview of the treatment schedule is displayed in **Figure 16** (for further specifics see the Study Schedule in Section 7.1).

**Figure 15 : EOCRC1-22 Overview trial design**



## 4.2 General trial design

There will be a 2-stage consent procedure for the trial, including a first consent for basic disease, age information, and HLA-typing (or the use of already available information in the patients' file; such information even if based on testing before the screening period is acceptable) since a large proportion of the patients (approximately half of the patients) will not match the HLA-A2 prerequisite to receive the HLA-A2 specific EO2040 immunization compound. The reason for the initial minimized consent (related to the procedure of HLA-testing, and before testing also establishing based on available information that the patient is eligible in relation to CRC type, prior therapy, and has an age ≥ 18 years) is to spare the necessity of all other testing for possible enrollment in the trial for patients who are not known to be HLA-A2 positive.

The second stage of the consent procedure will be related to treatment, and follow-up aspects of the trial procedures.

[redacted]

Study treatments will be administered until study treatment termination criteria as outlined in **Section 5.3.3** are fulfilled, e.g. reaching maximum study treatment period (6 months), unacceptable toxicity, or relapsing disease. At the time of stopping study treatment, appropriate other treatment will be advised by the treating physician on an individual patient basis. The patient should continue, even if study treatment is stopped, study follow-up measures for as long as the individual patient consent for follow-up is not withdrawn, the site is open, and the study not terminated per plan, or early, but for a maximum length of 3 years after start of study treatment.

All assessments of possible relapse in the trial will be determined at the sites by the principal investigators, or designees, according to the standard clinical criteria used at the site.

***Of note, there is no current knowledge publicly available regarding the potential of pseudoprogression at immunotherapy in patients with ctDNA defined MRD of CRC. Thus, at scanning, especially at the 3-month study timepoint, if a relapse is indicated by scanning it is recommended to, if at all possible, also document the relapse with histology or cytology.***

## 4.3 Trial Safety

The safety oversight of the trial is a combined effort of the investigators with their local study teams, the Independent Data Monitoring Committee (IDMC), and the Sponsor (also represented when applicable by designated CROs). The setup and tasks of the IDMC are outlined below (see Section 8.6), and details of the IDMC processes and procedures, are also outlined in a separate charter.

Pre-planned safety monitoring measures are outlined in the Study Schedule (see Section 7.1).

Safety profiles of the study treatments are outlined in Section 1.3.2 (EO2040), and Section 6.2.5 (nivolumab).

Administration of EO2040 in combination with nivolumab is described in Section 6.3, and treatment modifications are described in Section 6.4.

[redacted]

## 4.4 End of study

### *4.4.1 Planned study completion and study duration*

The clinical data cut-off for the final study analysis, assuming a positive outcome of Cohort 1 and recruitment to Cohort 2A, is planned to be 36 months after the last patient has been starting study treatment in Cohort 2A (or if this patient is not alive, when the next "last started patient" has been followed for 36 months after the start of study therapy).

The planned end of study, defined as the date of the last visit of the last patient in the study, is based on the foreseen clinical data cut-off per above.

[redacted]

Study sites will be closed upon decision (per plan or earlier) and once all patients at the site have completed the study (in line with decision to close study) and when all close out activities have been finalized. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed, except for the sites without any patients included which could be administratively closed by a close-out letter.

### *4.4.2 Early site closure and early study termination*

The Sponsor reserves the right to close a study site or terminate the study at any time for any reason at the sole discretion of the Sponsor.

Reasons for early study termination may include, but are not limited to:

- safety findings unknown to date (i.e. not previously reported in any similar investigational study drug trial with respect to their nature, severity, and/or duration), or significantly changed frequency, severity, or duration of known/anticipated/previously reported safety events,
- medical or ethical reasons affecting the continued performance of the study,
- difficulties in recruitment of patients,
- regulatory authority request, and

- cancellation of development of EO2040.

The Investigator may initiate study site closure at any time, provided there is reasonable cause, and adequate notice is given to the Sponsor in advance of the intended site closure.

Reasons for the early closure of a study site by the Sponsor may include, but are not limited to:

- failure of the Investigator to comply with the protocol, the requirements of the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) or local health authorities, the Sponsor's procedures, or Good Clinical Practice (GCP) guidelines, and
- inadequate recruitment of participants by the Investigator.

### 4.5 Discussion of 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 treatment setting as such, i.e. treatment of patients with CRC who after curative therapy have a ctDNA defined MRD, is by itself novel. However, detectable ctDNA after completion of curative therapies has nearly 100% positive predictive value for radiographic recurrence. Multiple studies demonstrate that the presence of ctDNA likely reflects radiographically unseen micrometastases, or MRD, which is predictive of recurrence and a useful screening tool for high-risk patients [21].

The lead-time of ctDNA positivity to radiographic recurrence is up to approximately 9 months in early-stage disease, which provides a window for evaluation of novel therapies in a setting for which there are no current standard-of-care guidelines [22].

Clearance of ctDNA is in the current study used as a surrogate endpoint to assess within the assumed window before there is a documented radiographic recurrence if the study treatment can impact this disease parameter. Standard outcome parameters as DFS and OS will also be followed.

[redacted]

The early exploratory nature of the trial in relation to the setting makes, in the view of the Sponsor, an open-label design adequate.

The design with two sequential cohorts starting with the assumed most efficacious cohort first (direct combination of EO2040 with nivolumab from treatment day 1; available experience from other microbiome-derived therapeutic vaccine trials indicate that the expansion of specific T cells is faster if the combination is used upfront, instead of delaying the anti-PD1 blockade) has been selected to as fast as possible evaluate if the treatment concept might impact, at all, ctDNA positivity. If the initial cohort, with the current maximum concept treatment, would have a positive outcome, a modification of study treatment will be done to evaluate the option that the microbiome-derived therapeutic

vaccine by itself, or with a delayed start of nivolumab, would also be efficacious. If this would be the case, one would potentially be able to spare patients the risk of some adverse events linked to nivolumab. Of note, neither the trial investigators, nor the Sponsor, considers it adequate to reverse the order of the cohorts since the setting argues for an approach directed towards maximum efficacy. Also, with the predetermined relatively short treatment duration it is assumed that the toxicity burden from nivolumab will be limited, and acceptable, considering the setting.

The trial includes, due to the specific characteristics of the combination of EO2040 and nivolumab key well defined strategies for patient management at possible immune-mediated reactions (see Section 6.4.1 and extended treatment algorithms in Section 12.5).

The efficacy assessments by scanning in the trial are based on site/local investigator assessments which is considered adequate for fast decisions regarding study treatments, and in context of key endpoints related to ctDNA testing.

# 5 POPULATION

## 5.1 Inclusion criteria

To be eligible to receive study treatment, a patient must meet all the criteria below:

1. Provided written informed consent prior to any study-related procedures (initial consent is for screening part #1 procedures, and a second consent is for screening part #2 procedures; see Section 7.2 and Section 7.3, respectively).
2. Histological confirmation of colorectal cancer (confirmation at initial diagnosis is sufficient).
3. Post R0-resection (i.e. microscopically margin-negative resection and no gross or microscopic tumor remaining in the primary tumor bed) of stages II, III, or IV CRC and completion of all planned standard of care perioperative and/or adjuvant therapies.

   [redacted]

4. Presence of minimal residual disease as defined by a positive ctDNA assay after completion of all planned standard of care therapies.

   [redacted]

5. Age ≥ 18 years old.
6. Human leukocyte antigen (HLA)-A2 positive.
7. No evidence of radiographic disease (computer tomography or magnetic resonance imaging; optimized to detect metastatic disease, e.g. with contrast as applicable) that requires immediate therapeutic intervention as assessed by the treating physician, within 28 days of (before or after) a positive ctDNA assay.

   [redacted]

   [redacted]

8. ECOG performance status 0 or 1 (see Section 12.1).

9. Female patients of childbearing potential must have a negative serum pregnancy test within 72 hours prior to start of study therapy.

10. Considering the embryofetal toxicity of the immune checkpoint inhibitor (ICI) shown in animals' models, the following recommendations for contraception must be followed:

    a. If not surgically sterile, female patients of childbearing potential age must use highly effective contraception from signing the Informed Consent Form (ICF) through 6 months after the last treatment dose administered. Highly effective contraception includes (according to Clinical Trial Facilitation Group: Recommendations related to contraception and pregnancy testing in clinical trials [104]):

        i. combined (estrogen and progesterone containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, transdermal,
        ii. progestogen-only hormonal contraception associated with inhibition of ovulation: oral, injectable, implantable,
        iii. intrauterine device (IUD),
        iv. intrauterine hormone-releasing system (IUS),
        v. bilateral tubal occlusion,
        vi. vasectomized partner, and
        vii. sexual abstinence when in line with the preferred and usual lifestyle of the patient (e.g. periodic abstinence is not considered a highly effective method).

    In each case of delayed menstrual period (over 1 month between menstruations), confirmation of absence of pregnancy is strongly recommended. This recommendation also applies to women of childbearing potential with infrequent or irregular menstrual cycles.

    b. If not surgically sterile, male with female partner of childbearing potential age must use condom from signing the ICF through 6 months after the last treatment dose administered. Males must ensure that their partners of childbearing potential use highly effective contraception also. [redacted]

12. Patients willing and able to comply with the scheduled visits, treatment plan, laboratory tests, and other study procedures indicated in the protocol.

### 5.2 Exclusion criteria

Patients who meet any of the following criteria will not be eligible to participate in the study:

1. Patients treated with dexamethasone > 2 mg/day or equivalent (i.e., 13 mg/day of prednisone) within 14 days before start of study therapy, unless required to treat an adverse event (AE).

[redacted]

2. Patients treated with radiotherapy within 12 weeks, and cytotoxic chemotherapy therapy within 28 days (or 5 half-lives of the compound(s) administered if longer) before study treatment start.
3. Patients with persistent Grade ≥ 2 toxicities (according to NCI-CTCAE v5.0). Toxicities must be resolved for at least 2 weeks to Grade 1 or less. However, alopecia, neuropathy, and other persisting toxicities not constituting a safety risk based on Investigator's judgment are acceptable.
4. Patients who have received any prior treatment with compounds targeting PD1, PD-L1, CTLA-4, or similar compounds where general resistance against therapeutic vaccination approaches might have developed.
5. Patients with the following abnormal laboratory values:
    a. Hemoglobin < 10 g/dL (6.2 mmol/L); transfusion is acceptable to reach the value.
    b. Absolute neutrophil count decrease (<1.5 x$10^9$/L).
    c. Platelet count decrease (< 75 ×$10^9$/L).
    d. Total bilirubin > 1.5 ×upper limit of normal (ULN; according to the performing laboratory's reference ranges); except participants with Gilbert Syndrome who must have a total bilirubin level of < 3.0 xULN.
    e. Alanine aminotransferase (ALT) > 3 ×ULN.
    f. Aspartate aminotransferase (AST) > 3 ×ULN.
    g. Serum creatinine increase (> 1.5 ×ULN).
    h. Abnormal thyroid function per local laboratory levels; note, patients with hypothyroidism only requiring hormone replacement therapy, and patients with long term (judged by the treating physician) stable antithyroid therapy due to hyperthyroidism, are permitted to enroll. Patients with abnormal thyroid laboratory values judged by the treating physician as clinically non-relevant are also eligible.
6. Patients with presence of other concomitant active, invasive malignancies that may interfere with ctDNA analysis (known clonal hematopoiesis of unknown potential allowed).
7. Patients with clinically significant active infection, cardiac disease, significant medical or psychiatric disease/condition that, in the opinion of the Investigator, would interfere with the interpretation of patient safety or study results or that would prohibit the understanding or rendering of informed consent (i.e. only consent able patients can be enrolled in the study) and compliance with the requirements of the protocol – including (but not limited to):
    a. Bacterial sepsis, COVID-19, or other similarly severe infections (clinical assessment is the basis for exclusion of severe infections; if clinical suspicion, adequate testing should be performed to exclude severe infections).
    b. New York Heart Association > Grade 2 congestive heart failure within 6 months prior to start of study therapy (see Section 12.2).
    c. Uncontrolled or significant cardiovascular disease, including:
        i. myocardial infarction within 6 months prior to start of study therapy,

ii. uncontrolled/unstable angina within 6 months prior to start of study therapy,

iii. diagnosed or suspected congenital long QT syndrome, and

iv. any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes).

d. Stroke within 6 months prior to start of study therapy.
e. Concurrent neurodegenerative disease.
f. Dementia or significantly altered mental status.

8. Patients with suspected autoimmune or active autoimmune disorder or known history of an autoimmune neurologic condition (e.g., Guillain-Barré syndrome).

[redacted]

9. Patients with a history of solid organ transplantation or allogeneic hematopoietic stem cell transplantation.
10. Patients with a history or known presence of tuberculosis.
11. Pregnant and breastfeeding patients.
12. Patients with a history or presence of human immunodeficiency virus (HIV) and/or active hepatitis B virus (HBV)/hepatitis C virus (HCV).
13. Patients who have received live or attenuated vaccine therapy used for prevention of infectious diseases including seasonal (influenza) vaccinations within 4 weeks of the first dose of study drug.
14. Patients with a history of hypersensitivity to any excipient, or active substance, present in the pharmaceutical forms of applicable study treatments.
15. Patients under treatment with immunostimulatory or immunosuppressive medications, including herbal remedies, or herbal remedies known to potentially interfere with major organ function.
16. Patients who have received treatment with any other investigational agent, or participation in another clinical trial (clinical trial including active interventions; participation in clinical trials for data collection purposes only are permitted) within 28 days (or 5 half-lives if longer) prior to start of study therapy. Note, during participation in the current study, parallel participation in other interventional studies is not allowed (participation in clinical trials for data collection purposes only are permitted; also, in the follow-up parts of interventional studies).

## 5.3 Removal of patients from therapy or study

### *5.3.1 Patient withdrawal of consent*

Patients may voluntarily withdraw from the study treatment, or the study as such, including procedures, at any time at his/her request for any reason.

[redacted]

### *5.3.2 Screen failures*

Screen failures are defined as patients who consent to participate in the clinical study but are not subsequently treated with study drug(s) due to failure of the eligibility criteria. [redacted]

### *5.3.3 Criteria for treatment discontinuation*

Patients should stop study treatment for any of the following reasons:

- reached maximum defined study treatment period (EO2040 in combination with nivolumab 6 months; see specifics in Study Schedule Section 7.1),
- intolerable toxicity in relation to study treatment and adverse events requiring treatment discontinuation according to this protocol (see Section 4.3 and Section 6.4, respectively),
- pregnancy,
- confirmed tumor relapse by scanning,
- any medical condition which may jeopardize the patient's safety if he/she continues trial treatment,
- major protocol violation (including non-compliance to study schedule by the patient) judged to significantly impact safety of the patient or interpretability of the trial,
- decision by the Investigator, Sponsor, or any regulatory authority, that treatment discontinuation is in the best interest of the patient,
- patient withdrawal of consent to receive further study treatment, and
- planned or early termination of the study (see Section 4.4).

### *5.3.4 Lost to follow-up*

A patient will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a patient fails to return to the clinic for a required study visit:

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible, counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether, or not, the patient wishes to and/or should continue in the study.

In cases in which the patient is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the patient (where possible, 3 telephone calls, and if necessary, a certified letter to the patient's last known mailing address, or local equivalent method). These contact attempts should be documented in the patient's medical records.

#### *5.3.5 Criteria for study participation termination*

Reasons for patient withdrawal from the study may include, but are not limited to:

- patient withdrawal of consent for any further trial procedures (see also Section 5.3.1),
- patient lost to follow-up,
- major protocol violation (including non-compliance to study schedule by the patient) judged to significantly impact safety of the patient or interpretability of the trial,
- decision by the Investigator, Sponsor, or any regulatory authority, that study participation discontinuation is in the best interest of the patient,
- planned or early termination of the study (see Section 4.4).

#### *5.3.6 Follow-up of patients discontinued from study treatment or withdrawn from study*

The primary reason for study treatment discontinuation should be documented in the eCRF. At treatment discontinuation, patients will complete follow-up visits according to Section 7.5. [redacted]

In cases of withdrawal from the study, every effort should be made to obtain adequate information. The primary reason for withdrawal from the study should be documented in the eCRF. If possible, patients will complete a follow-up visit according to Section 7.5.

In cases with complete informed consent withdrawal (see also Section 5.3.1), patients will not be followed for any reason, although date of death will be recorded, where applicable and possible to retrieve.

#### *5.3.7 Procedures for handling incorrectly enrolled patients*

If a patient is found to have been incorrectly enrolled in the study, the Sponsor must be notified as soon as the error is discovered. The Sponsor will decide on the appropriate action to take regarding study treatment and continuation of the patient in the study; if applicable the IDMC should be consulted.

#### *5.3.8 Patient replacement*

Generally, no replacement is foreseen. [redacted]

# 6 TREATMENT OF PATIENTS

## 6.1 Description of EO2040

### *6.1.1 Pharmaceutical form*

The Investigational Medicinal Product (IMP) of the study, EO2040 Drug Product (DP), [redacted] containing 3 peptide drug substances (EO2317, EO2318, and UCP2). [redacted] final concentration of each peptide in the [redacted] DP in adjuvant is 300 µg/mL.

Further details regarding the components of EO2040 can be found in Section 1.3**,** and in the current version of the EO2040 IB.

### *6.1.2 Preparation of the emulsion*

The IMP is provided to the clinical sites [redacted]

Detailed composition of EO2040 is presented in **Table 5**.

[redacted]

**Table 5 : Composition of the final emulsified EO2040 DP in adjuvant obtained by reconstitution**

[redacted]

- [redacted]
- [redacted]
- Regarding additional material see Section 6.1.3.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

### *6.1.3 Packaging and labelling*

EO2040 will be manufactured as per Good Manufacturing Practice (GMP) and supplied by the Sponsor [redacted]

- [redacted]
- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

[redacted] A complete record of batch numbers and expiry dates of all study drugs and labels will be maintained in the Sponsor's study file.

A detailed process for ordering the IMP, handling and administration, packaging, and labeling the IMP will be specified in the Pharmacy Manual.

#### *6.1.4 Storage and stability*

The study treatment must be stored in a secure limited access area according to local regulations. [redacted]

### 6.2 Description of nivolumab

#### *6.2.1 Pharmaceutical form*

Nivolumab is sterile concentrate solution for infusion administration. The solution is clear to opalescent, colorless to pale yellow liquid that may contain a few light particles. The solution has a pH of approximately 6.0 and an osmolality of approximately 340 mOsm/kg.

List of excipients include; sodium citrate dihydrate, sodium chloride, mannitol (E421), pentetic acid (diethylenetriaminepentaacetic acid), polysorbate 80, sodium hydroxide (for pH adjustment), hydrochloric acid (for pH adjustment), water for injections.

Further details regarding nivolumab can be found in the European SmPC or the US PI for nivolumab [90, 91].

#### *6.2.2 Preparation of the formulation*

Instructions for preparation of nivolumab for infusion can be found in the European SmPC or the US PI for nivolumab [90, 91].

#### *6.2.3 Packaging and labeling*

Nivolumab will be obtained from commercial sources by the Sponsor and clearly labeled for use within the trial.

Adequate supplies of nivolumab in 24 mL glass vials will be provided to each investigational site for preparation and infusion. The vial stopper will be a coated butyl rubber with a grey aluminum flip-off seal.

#### *6.2.4 Storage and stability*

Nivolumab should be stored in the refrigerator at 5 ± 3°C in its original package in order to protect from light. Once opened, the medicinal product should be infused or diluted, and infused immediately (99, 100).

If not used immediately, chemical and physical in-use stability of nivolumab has been demonstrated for 24 hours at 5 ± 3°C protected from light and a maximum of 8 hours at 20°C

to 25°C with room light (this 8-hour period of the total 24 hours should be inclusive of the product administration period).

### *6.2.5 Expected safety profile of nivolumab*

The following summary is based on the European SmPC [91].

In the pooled dataset of nivolumab as monotherapy across tumor types (n = 3771) with minimum follow-up ranging from 2.3 to 28 months, the most frequent adverse reactions (≥ 10%) were fatigue (46%), musculoskeletal pain (31%), diarrhea (26%), nausea (24%), cough (24%), rash (24%), dyspnea (18%), pruritus (18%), decreased appetite (18%), constipation (17%), abdominal pain (16%), upper respiratory tract infection (16%), arthralgia (15%), pyrexia (14%), vomiting (14%), headache (13%) and oedema (11%). The majority of adverse reactions were mild to moderate (Grade 1 or 2). With a minimum of 63 months follow-up in NSCLC, no new safety signals were identified.

In addition, nivolumab is associated with immune–related adverse reactions. With appropriate medical therapy, immune-related adverse reactions resolved in most cases. **Table 6** presents the percentage for immune-related adverse reactions leading to permanent discontinuation of nivolumab and the percentages requiring high-dose corticosteroid dosing.

For the laboratory abnormalities observed with nivolumab monotherapy, please refer to the European SmPC or to the US PI [90, 91].

Of the 3224 patients who were treated with nivolumab monotherapy 3 mg/kg or 240 mg every 2 weeks and evaluable for the presence of anti-product-antibodies, 286 patients (8.9%) tested positive for treatment-emergent anti-product-antibodies with sixteen patients (0.5%) testing positive for neutralizing antibodies.

Generally, no overall differences in safety regarding nivolumab were reported between elderly (≥ 65 years) and younger patients (< 65 years). In the nivolumab non-squamous non-small cell lung cancer study (CA209057), the safety profile in patients with baseline renal or hepatic impairment was comparable to that in the overall population. These results should be interpreted with caution due to the small sample size within the subgroups.

**Table 6 : Nivolumab immune-related adverse reactions leading to permanent discontinuation or requiring high-dose corticosteroids**

| | Nivolumab 3 mg/kg or 240 mg monotherapy % |
|---|---|
| **Immune-related adverse reaction leading to permanent discontinuation** | |
| Pneumonitis | 1.4 |
| Colitis | 1.0 |
| Hepatitis | 0.9 |
| Nephritis and renal dysfunction | 0.2 |
| Endocrinopathies | 0.3 |
| Skin | 0.6 |
| Hypersensitivity/Infusion reaction | 0.1 |
| **Immune-related adverse reaction requiring high-dose corticosteroids[a,b]** | |
| Pneumonitis | 67 |
| Colitis | 13 |
| Hepatitis | 20 |
| Nephritis and renal dysfunction | 24 |
| Endocrinopathies | 7 |
| Skin | 3 |
| Hypersensitivity/Infusion reaction | 18 |

a: At least 40-mg daily prednisone equivalent.
b: Frequency is based on the number of patients who experienced the immune-related adverse reaction.

### 6.3 Administration of EO2040 and the combination of EO2040 and nivolumab

*Note, detailed instructions are also specified in the Pharmacy Manual.*

- EO2040 should be injected [redacted] from the start of the reconstitution [redacted]
- EO2040 [redacted] should be administered subcutaneously (SC) in a rotating way by injection (thus, an injection site will be used every fourth time), with the injection sites being right and left upper extremity and right and left lower extremity/inguinal area, with the more specific locations being:

  - For upper extremity = SC injection in the area of armpit, or deltoid muscle of arm, depending on the constitution of the patient.
  - For lower extremity = SC injection in the area of groin, or anterolateral thigh muscle, depending on the constitution of the patient.

- EO2040 will during the initial 6 weeks be administered SC 3 times, each administration followed by a 2-week observation period. After the initial 6 weeks EO2040 will be administered with a 4 weekly interval starting from the administration at week 7. See also Study Schedule in Section 7.1**.**
- All patients should be monitored for [redacted] after an EO2040 administration.
- Dose modifications for EO2040 include withholding of doses and treatment discontinuation (see Section 6.4).
- At combination treatment when EO2040 and nivolumab are to be administered on the same day, the order of administration is to start with EO2040, followed by a [redacted] after which the nivolumab infusion should be administered intravenously (IV) according to local guidelines. The dose of nivolumab should be 240 mg at visit 1 (week 1), visit 2 (week 3), and visit 3 (week 5), and from visit 4 (week 7) and onwards 480 mg per administration every 4 weeks. Observation after nivolumab administration should follow local guidelines.
- The only adjustments regarding dose for nivolumab are withholding of one or several doses, or discontinuation of treatment; such measures should be taken in accordance with the labeled recommendations for nivolumab in the European SmPC or the US PI [90, 91]; see also Section 6.4.
- In case of infusion-related reactions and hypersensitivity reactions, please refer to Section 12.4.
- In case of immune-related adverse events related to EO2040 and/or nivolumab, instructions for treatment modifications as outlined in Section 6.4 should be followed.

### 6.4 Treatment modifications for EO2040 and nivolumab

- [redacted]

[redacted]

[redacted]

[redacted]

- [redacted]

[redacted]

In case of immune-related adverse events related to EO2040 and/or nivolumab, instructions for treatment modifications as outlined in Section 6.4.1 should be followed.

General criteria for treatment discontinuation and study participation termination are outlined in Section 5.3.

### *6.4.1 Recommended treatment modifications for EO2040 and nivolumab in case of immune-related adverse reactions*

For suspected immune-related adverse reactions, adequate evaluation should be performed to confirm etiology or exclude other causes. Based on the severity of the adverse reaction, the treatment should be withheld, and corticosteroids administered. If immunosuppression with corticosteroids is used to treat an adverse reaction, a taper of at least 1-month duration should be initiated upon improvement. Rapid tapering may lead to worsening or recurrence of the adverse reaction. Non-corticosteroid immunosuppressive therapy should be added if there is worsening or no improvement despite corticosteroid use. **Table 7** shows the recommended treatment management for different immune-related adverse reactions. Regarding management of immune-related adverse reactions due to nivolumab see also the European SmPC or US PI [90, 91].

Regarding nivolumab, patients should be monitored continuously (at least up to 5 months after the last dose) as an adverse reaction with nivolumab may occur at any time during or after discontinuation of therapy. [redacted]

Patients will be reviewed on a case-by-case basis, should they need to discontinue EO2040 and/or nivolumab due to toxicity. See Section 6.4, regarding guidance for continuation of individual components of the used compounds in this trial.

In case of detected, or suspicious, immune-related adverse reactions it is recommended that the treating physician consult at least one of the following guidance documents for a thorough description regarding possible workup for final diagnosis:

- Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology 2017; 28 (Supplement 4): i119–i142 [105]

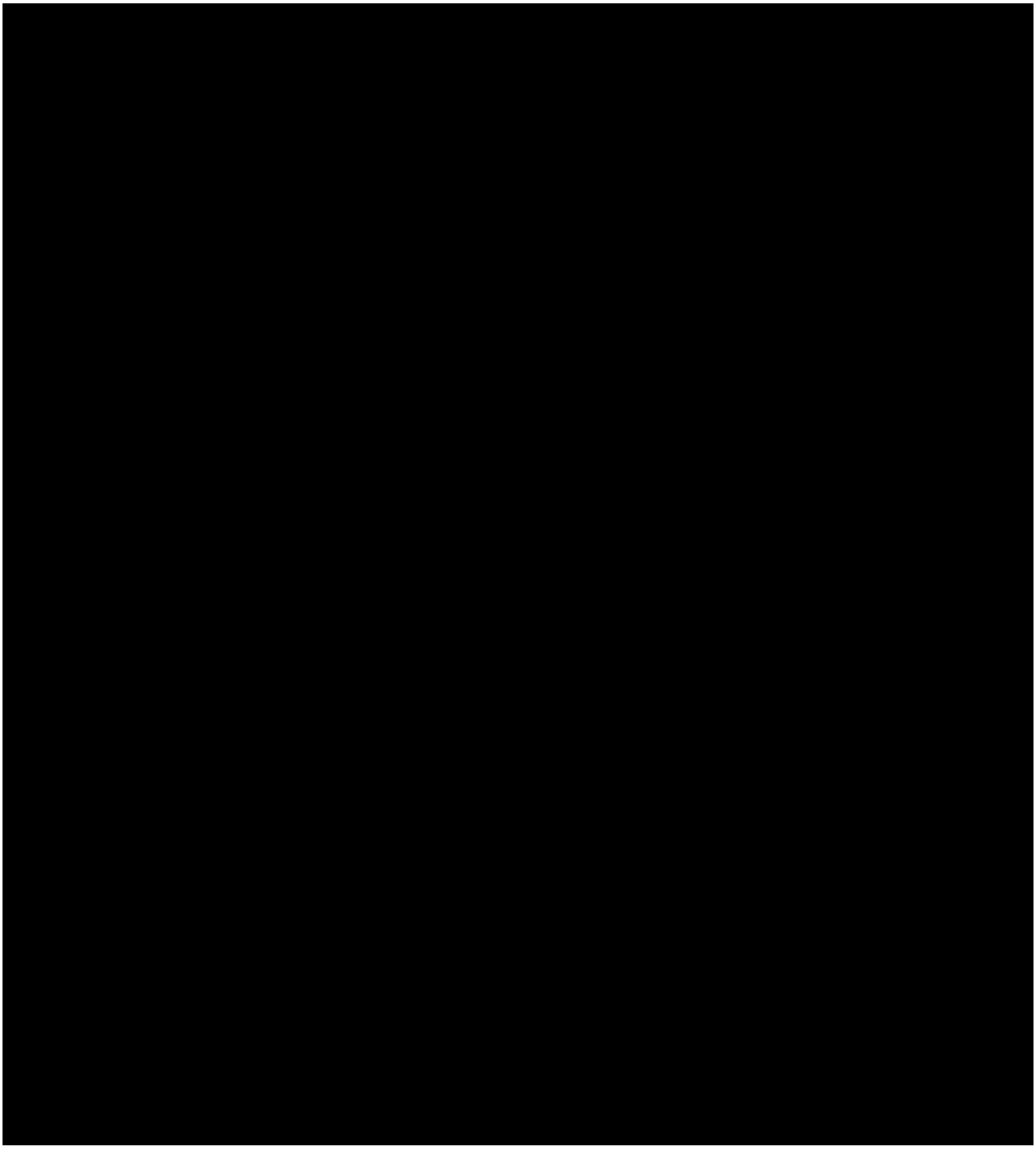
- Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: ASCO Guideline Update. J Clin Oncol 2021; 39 (36): 4073-4126 [106]

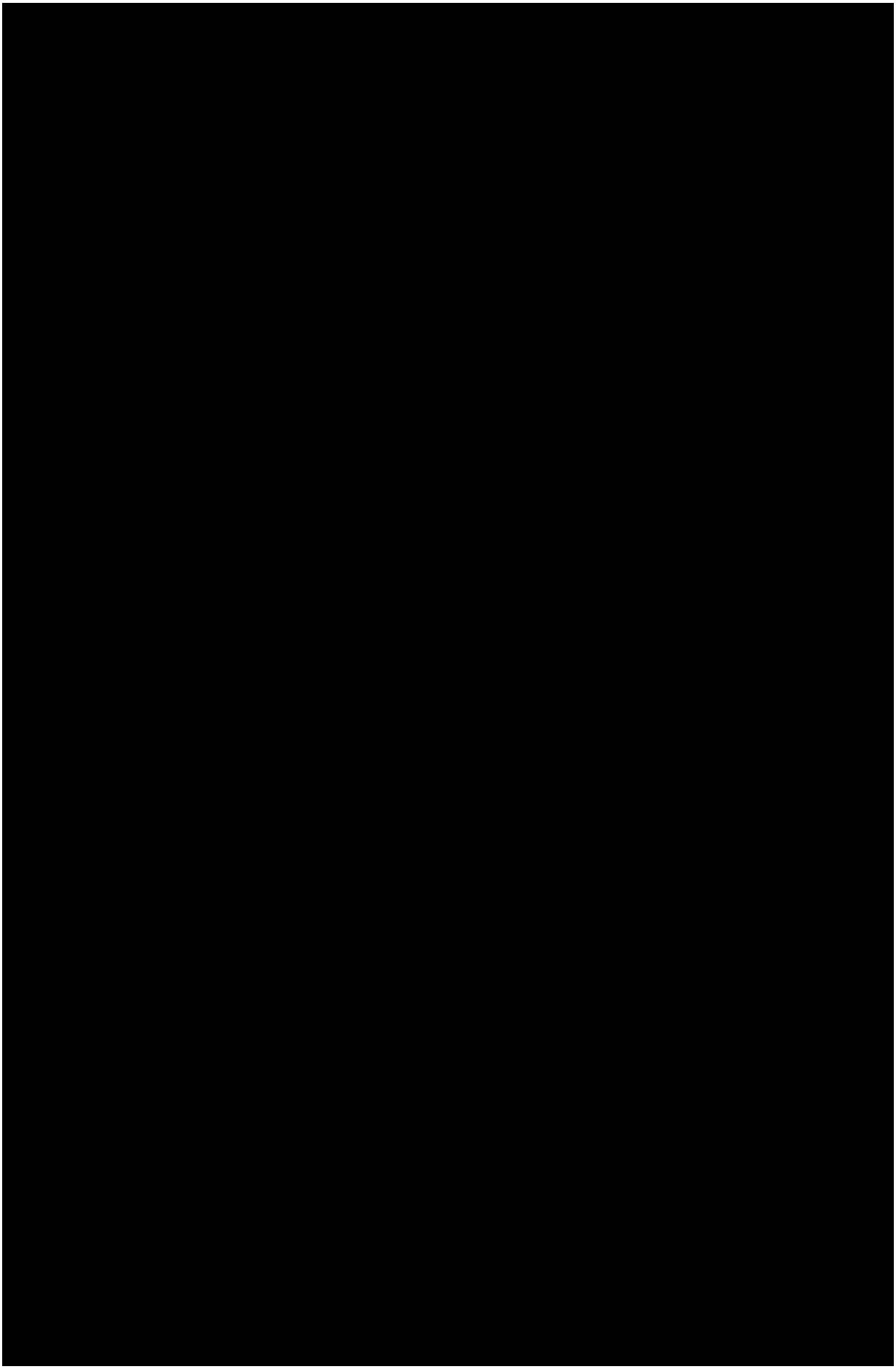
- NCCN Clinical Practice Guidelines in Oncology: Management of Immunotherapy-Related toxicities. Version 4.2021 - September 27, 2021.

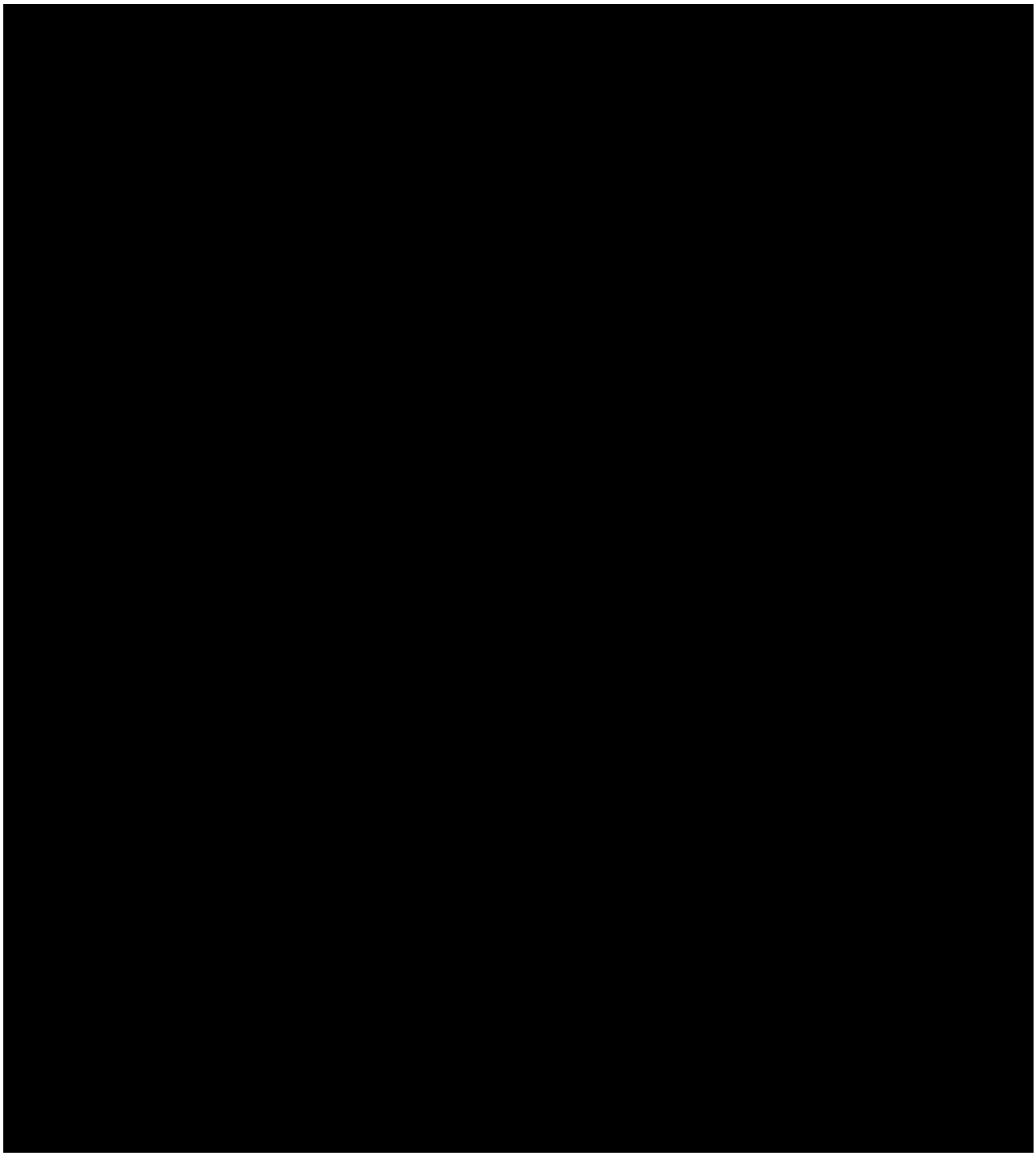
https://www.nccn.org/professionals/physician_gls/pdf/immunotherapy.pdf. Accessed on January 16, 2022 [107]

The documents indicated above also includes specific guidance regarding specific treatment of diagnosed immune-related adverse reactions (e.g. substitution therapies with hormones, recommended local or systemic steroid doses etc. in context of the different and varied reactions possible) which is recommended as a basis for treatment decisions [105, 106, 107]. In addition, the European SmPC and the US PI also includes treatment recommendations for some immune-related immune reactions [90, 91], and Section 12.5 outlines management algorithms regarding nivolumab for some specific adverse events.

**Table 7 : Recommended treatment modifications for EO2040 and nivolumab in relation to immune-related adverse reactions**







### 6.5 Study treatment accountability, reconciliation, and return

The Sponsor will be responsible for ensuring that the quality of the study treatment is adequate for the duration of the study.

It is the responsibility of the Investigator or designee to ensure that the study treatment is only dispensed to the adequate patient. The study treatment must be dispensed from official study sites by authorized personnel according to local regulations. The Investigator or designee must maintain accurate records of the study treatment receipt, dispensing information, and disposition.

Upon completion and termination of the study, all unused and/or partially used study treatment must be returned to the Sponsor or other authorized party. If return to Sponsor not

authorized by the Sponsor, study treatment shall be destroyed at the site following written confirmation by the Sponsor.

If study treatment is to be destroyed at the site, it is the Investigator's or designee's responsibility to ensure that arrangements have been made for disposal, drug accountability has been completed by the site monitor, procedures for proper disposal have been established according to applicable regulations, guidelines, and institutional procedures, and appropriate records of the disposal have been documented and provided to the Sponsor or designee.

All study treatments returned to the Sponsor or other authorized party must be accompanied by the appropriate documentation and be clearly identified. Study treatment may only be returned after drug accountability is completed. Returned supplies should be in their original containers (component vials that have clinical labels attached).

Empty vials should not be returned to the Sponsor. Empty vials may not be destroyed until drug accountability is completed. It is the Investigator's responsibility to arrange disposal of all empty vials according to the institutional regulations.

The return or destruction of unused study treatment should be arranged by the site monitor. Further guidance and information for the final disposition of unused study treatment are provided in the Pharmacy Manual.

## 6.6 Method of treatment assignment

This is an open-label (i.e. no blinding procedure will be implemented), non-randomized trial and each newly enrolled patient (upon signing the ICF) will be assigned a unique patient identification number (identifying trial, site, and patient), and assigned to the treatment after approval of eligibility by the Sponsor Medical Monitor according to the separate Cohort Administration Charter.

[redacted]

## 6.7 Ancillary treatments

### *6.7.1 Prior and concomitant treatments and procedures*

Prohibited treatments prior to trial enrollment are outlined in the exclusion criteria (see Section 5.2).

The Investigator must record the use of all prior and concomitant treatments and procedures, including medications and vaccines, taken during 28 days prior to signing informed consent. Anti-CRC treatments and procedures will be recorded as accurately as possible for the time from the diagnosis of the disease, i.e. for a longer time than 28 days prior to signing the informed consent. Likewise, for the duration of the study both prescribed and "over-the-counter" medications, including herbal remedies, should be recorded in the source documents and eCRF along with:

- reasons for use,
- dates of administration including start and end dates, and
- dosage information, including dose, frequency, and route of administration.

In case of surgical procedures, or other relevant non-drug medical interventions and procedures, all details must be collected in the eCRF in a similar manner as for medications per above.

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Patients should be discouraged from starting any new medication, both prescribed and over-the-counter, including herbal remedies, without consulting the Investigator unless the new medication is required for emergency.

### *6.7.2 Permitted concomitant medications*

All non-cancer therapies that the Investigator feels appropriate are allowed in this study, except for the medications outlined in protocol Section 6.7.3. Thus, patients should receive full supportive care during participation in the trial and standard of care treatment per local practice and according to the judgment of the Investigator or treating physician.

Administration of steroids through a route known to result in a minimal systemic exposure (topical, intranasal, intro-ocular, or inhalation) is acceptable.

Of note, the use of systemic corticosteroids and other immunosuppressants (beside trial treatment) should be avoided because of their potential interference with the pharmacodynamic activity of the investigational treatments. However, the use of systemic corticosteroids and other immunosuppressants might be of vital importance for patient safety in relation to treatment of adverse reactions (see Section 6.4 and Section 12.5). Also, steroid medication in relation to administration-related reactions might be vital (see Section 12.4).

The following is general guidance regarding steroid treatment:

- If possible, restrict use of systemic steroids to a dexamethasone dose < 2 mg/day or equivalent (i.e. 13 mg/day of prednisone, or 53 mg/day of hydrocortisone), unless required to treat an adverse event (see above).
- Adrenal replacement steroid doses > 2 mg/day dexamethasone (i.e. 53 mg/day of hydrocortisone) is permitted in patients needing steroid replacement therapy but should be discussed with the Sponsor.

In any case of steroid administration, it is of special importance to record dosage, frequency, dates of administration, and route of administration in the eCRF.

**Recommendations regarding vaccination against SARS-CoV-2/COVID-19**

- The Sponsor recommends strongly that patients if possible are vaccinated against SARS-CoV-2/COVID-19 before inclusion into the study. If vaccine is available for only one vaccine administration before study inclusion this is also recommended, and in such cases the timing of the second vaccine administration (and potential further doses) are to be discussed and agreed with the Medical Monitor. The recommended time interval between a COVID-19 vaccine administration and the initial study drug administration is 2 weeks, however, other trial exclusion criteria should also be considered (e.g. regarding

patients with persistent toxicities) when assessing possible timing of start of study treatment in relation to COVID-19 vaccine administration.

- For patients who have not been vaccinated against SARS-CoV-2/COVID-19 before study inclusion, but vaccine becomes available for them during their study participation, or a booster dose of SARS-CoV-2/COVID-19 vaccine is deemed necessary, the Sponsor also strongly recommends vaccination. For such patients the Sponsor recommends administration of the first dose of the COVID-19 vaccine during the boosting phase (EO2040 administered every 4 weeks), 2 weeks after the latest EO2040 administration (and thereby 2 weeks before the next EO2040 administration), and in a different location *[Note, if this time schedule would lead to unnecessarily long waiting time for COVID-19 vaccination, alternative administration time points should be discussed with the Medical Monitor]*.
- The currently (2021-03-24) by US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved COVID-19 vaccines are acceptable for use in the trial (local restrictions per country specific authority of course takes precedence):

| **COVID-19 Vaccine developer/ manufacturer** | **Vaccine platform** | **Type of candidate vaccine** | **Number of doses (base)** | **Timing between doses*** | **Route of administration** |
|---|---|---|---|---|---|
| University of Oxford/AstraZeneca | Non-Replicating Viral Vector | ChAdOx1-S | 2 | 28 days | IM |
| Moderna/NIAID | RNA | LNP-encapsulated mRNA | 2 | 28 days | IM |
| BioNTech/Fosun Pharma/Pfizer | RNA | 3 LNP-mRNAs | 2 | 21 days | IM |
| Johnson & Johnson/ Janssen | Non-Replicating Viral Vector | Ad26.COV2-S | 1 | NA | IM |

* Local country/site timing between doses might be different from what is stated in the table.

### *6.7.3 Prohibited medications and other therapies*

The use of systemic corticosteroids and other immunosuppressants should be avoided because of their potential interference with the pharmacodynamics activity of the study treatment. However, in some cases, systemic corticosteroids cannot be avoided; guidance regarding steroid use for patients participating in the trial is therefore outlined in Section 6.7.2.

The use of live or attenuated vaccine therapy for prevention of infectious diseases during treatment with study medication and for at least 3 months after end of treatment with study medication should be avoided. If there is no alternative, to live or attenuated, vaccine available, and it is considered of utmost importance for the individual patient to receive the

vaccine, an individual benefit/risk assessment should be made regarding the duration of "wash-out" period necessary after termination of study treatment, and study treatment should be stopped as applicable.

Patients should be discouraged from taking medications, including herbal remedies, with immunostimulatory properties, or known to potentially interfere with major organ function, during trial participation. If needed, any such possible therapy should be discussed between the treating physician and the Medical Monitor of the Sponsor.

During trial participation patients should not receive any other anti-CRC treatment than the specified trial treatments, and treatments outlined in Section 6.7.2.

If any other anti-CRC treatment, or procedure, would be considered for start in an individual patient, it is the duty of the Investigator to discuss the consideration with the Medical Monitor of the Sponsor for a decision on how to handle continued trial specific treatment and trial participation.

Start of non-trial systemic anti-CRC treatments would lead to censoring of the patient for relapse related endpoints at the time of start of the therapy; or assessment of the patient as having relapsed disease if relevant criteria would be fulfilled.

#### *6.7.4 Contraception*

Potential postmenopausal status for female patients will be confirmed with a screening serum follicle-stimulating hormone (FSH) level > 40 mIU/mL. Rules for contraception during study participation are outlined in the inclusion criteria, see Section 5.1.

### 6.8 Treatment compliance

Since all study related treatments are administered at the clinical site, the Investigator or designee must maintain accurate records of all study treatments, including as applicable dates of study drug receipt, quantities received and dispensed, and batch/lot numbers. In addition, study related treatments must be noted in the patient's medical records and eCRF, with the date and time of administration and dose of each study treatment, alternatively information regarding dispense documented. Based on documentation per above treatment compliance is going to be monitored and reported.

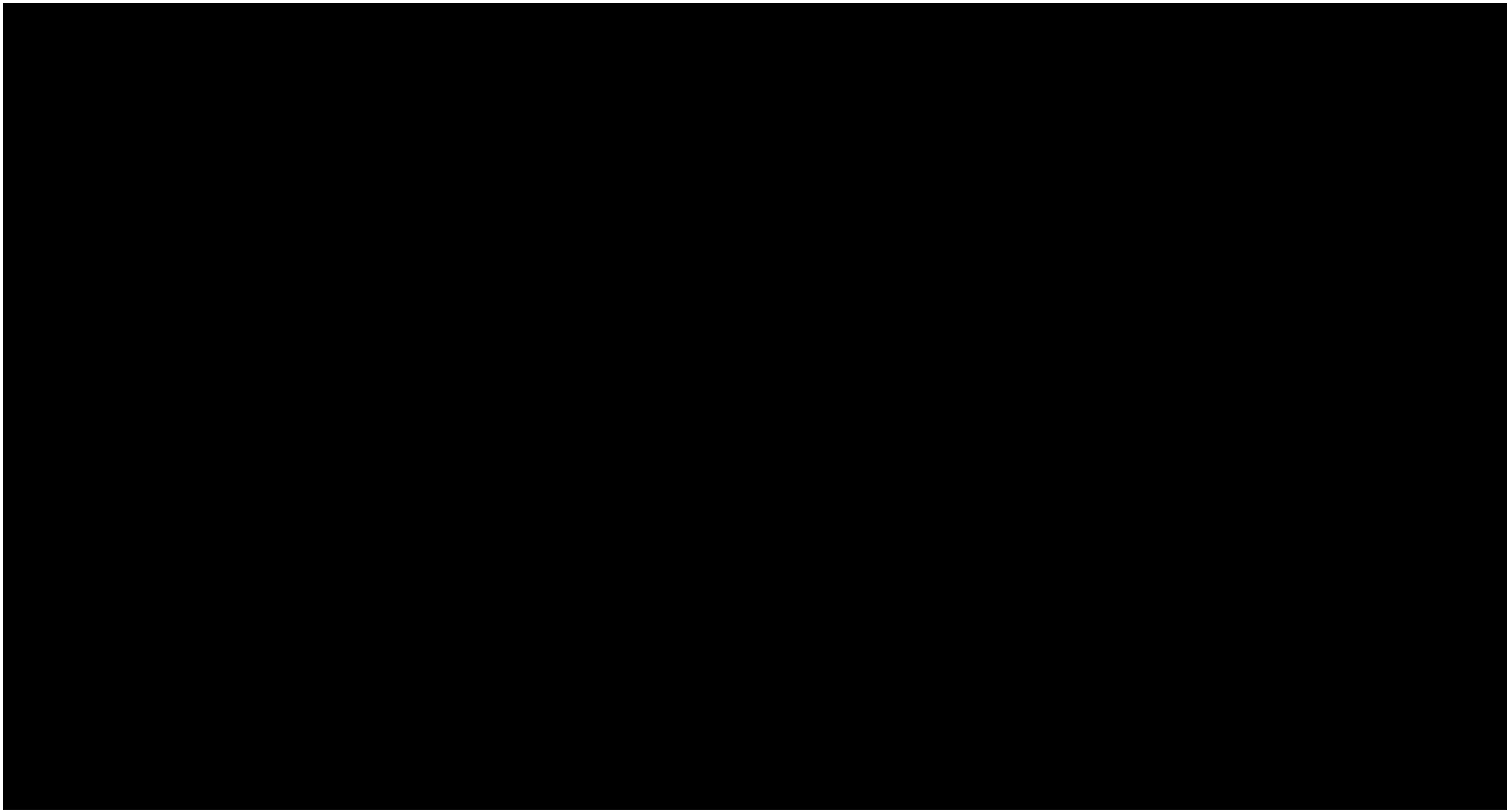
# 7 STUDY ASSESSMENTS AND PROCEDURES

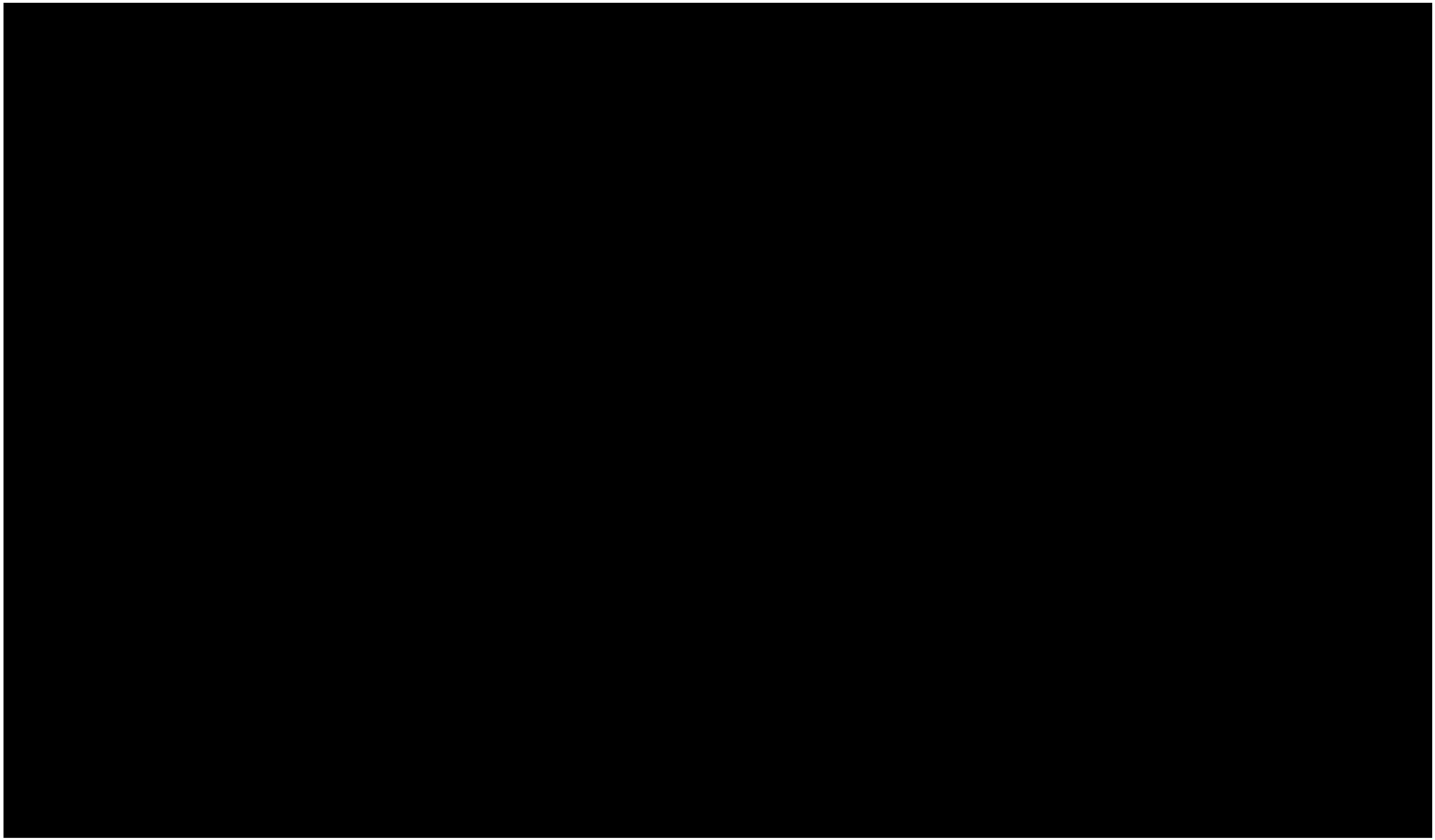
## 7.1 Study schedule

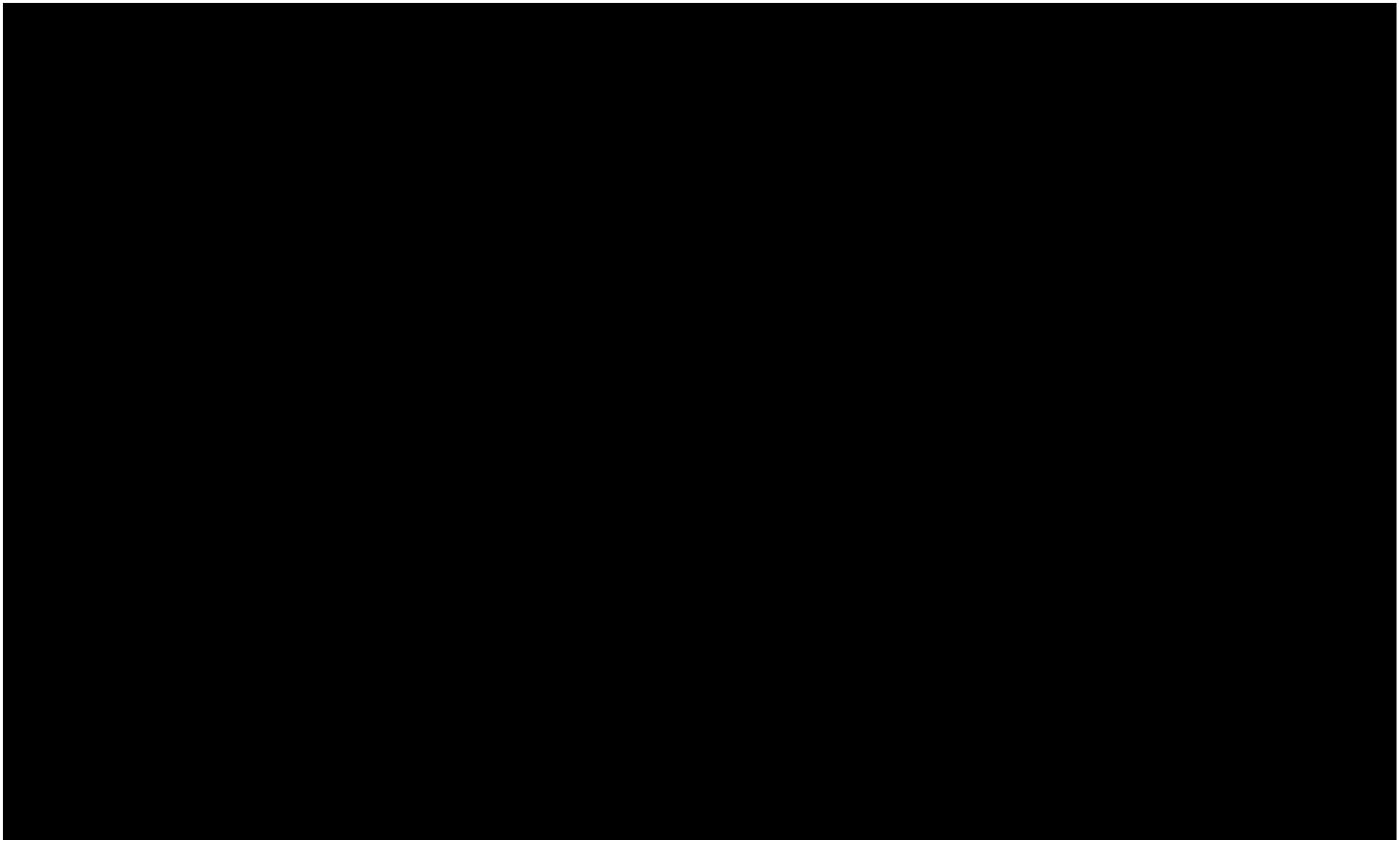
Study assessments and procedures, and their timings (including visit windows) are summarized in the Study Schedule (**Table 8**). As protocol exemptions are not foreseen in this trial, except in case of immediate safety concerns, any deviation from the planned study schedule should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the patient should continue, or discontinue, the study treatment. Adherence to the study design requirements, including those specified in the Study Schedule, is essential and required for adequate study conduct.

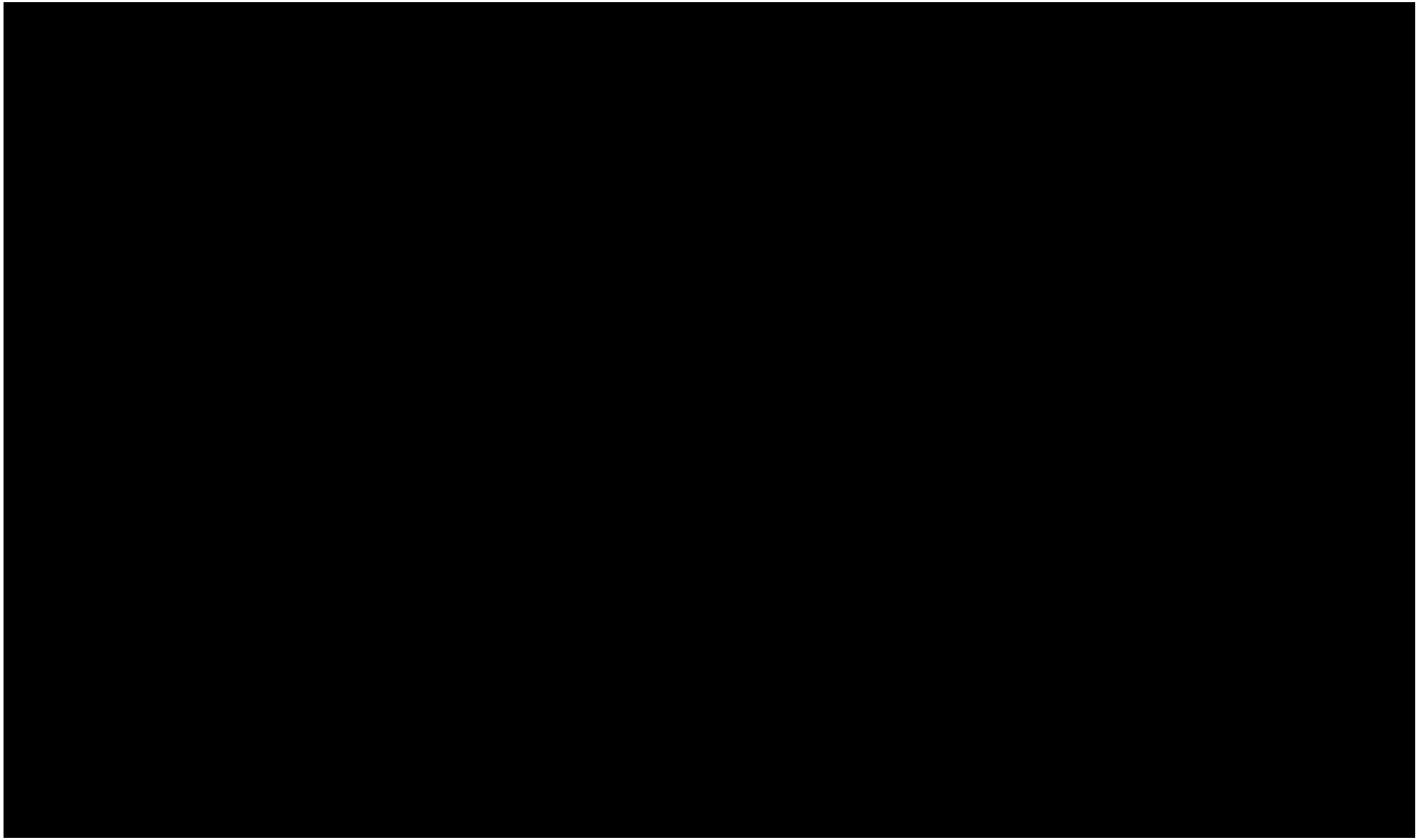
Note, for safety reasons, unscheduled visits, and unscheduled investigations, in the best interest of the patient per judgement of the treating physician might be instituted at any time during trial participation for an individual patient.

**Table 8 : Study Schedule**









### 7.2 Screening period #1

The trial will include a 2-stage consent and screening procedure (see Section 4.2).

The initial minimized consent is mainly related to the procedure of HLA-testing, and before testing also establishing, based on available information, that the patient has eligible CRC, and an age ≥ 18 years (inclusion criteria #1-7; see Section 5.1). Abbreviated medical history means collection of history of CRC, including treatment history, at a detailed enough level to enable assessment of inclusion criteria #2-7 (see Section 5.1).

The following will be done during screening period #1:

- study ICF procedure for consent #1, and
- eligibility ascertained for inclusion criteria #1 to #7; for the disease this means to ascertain that the patient has eligible CRC, and an age ≥ 18 years, plus HLA-testing.

HLA-testing can be performed within the scope of the trial, but HLA-information based on testing before the screening period is also acceptable (no time limit is applied; patients with prior allogeneic hematopoietic stem cell transplantation are not eligible for the trial).

HLA-testing will be performed locally; information regarding the local lab, including methods used will be collected.

Low resolution HLA typing, i.e. typing of the HLA A loci 2 to 2 digits is sufficient for trial screening purposes, but 4 digits resolution (high resolution) typing of the HLA A loci 2 should be conducted, if at all possible (but is not completely mandatory – see further below), on all patients who participate in the trial.

If high resolution testing is slower (to get a response) than low resolution testing, a possibility is thus to first make a test with low resolution for trial screening, and then at a later point in time (or on the same sample if possible) also make a high-resolution testing. There is a local site judgment, in relation to other screening procedures, to be made regarding what is “too slow” in relation to utilizing low- or high-resolution testing; but as a general guidance it is assumed that a result for HLA testing for screening purposes should be available within approximately a week. If for local reasons it is not possible to manage high resolution testing (e.g. due to sampling restrictions, testing abilities, or anything else), either for screening, or at a later point in time (per above), it is acceptable with low resolution testing only.

All information available regarding the individual patient MHC-composition will be collected, i.e. also information beside the HLA-A type. Patients who are not HLA-A2 positive will be assigned a “screen failure”-status and not continue any further trial procedures; such patients will be replaced.

### 7.3 Screening period #2

The second stage of the consent procedure, and screening period #2, will be related to trial treatment and follow-up aspects of the trial procedures, except those outlined in Section 7.2 (for remaining inclusion/exclusion criteria see Section 5). All timelines included in the protocol and related to the signature of the ICF are referring to the signature of this second part of the ICF. All evaluations must be completed and reviewed within the timeframe defined in the Study Schedule (**Table 8**) to confirm that potential patients meet all eligibility criteria. The Investigator will record adequate details of all patients screened and to confirm eligibility, or record reasons for screen failure, as applicable (see Section 5.3.2).

Procedures conducted as part of the patient's routine clinical management (e.g. blood counts) and obtained before signing the consent form may be utilized for screening purposes, provided the procedure met the protocol-specified criteria. Results obtained also the week before screening period #2 started (i.e. from study day -35 per the Study Schedule) can be used for study purposes provided approved by the study Medical Monitor.

All screening examinations will be performed locally, at the site or an affiliate site due to e.g. the location of the site and the patient.

The maximum amount of blood to be taken from each patient at screening, or over the duration of the study, including any extra assessments that may be required, will not exceed volumes as stated by local regulations. Repeat, or unscheduled samples, may be taken for safety reasons, or for technical issues with the samples.

Patients who do not fulfill all eligibility criteria will be assigned a "screen failure"-status and not continue any further trial procedures; such patients will be replaced.

The following will be done during screening period #2:

- study ICF procedure for consent #2,
- eligibility ascertained for inclusion and exclusion criteria (see Section 5),
- documentation of
    - patient demographics (including race and ethnicity),
    - extended history of hematology/oncology disease(s), including dates of diagnosis and treatments history, and especially for all relevant information for adequate grade, stage, and prognostic system evaluations of the CRC,
    - medical history of other (than hematology/oncology) disease processes (all prior significant illnesses that are relevant to patient safety or that the patient has experienced prior to screening; especially history of anaphylaxis or other reactions following vaccination, and history of immune deficiencies), and concomitant illness (illnesses present at the time of informed consent are to be regarded as concomitant illnesses; illnesses first occurring or detected during the study and/or worsening of a concomitant illness during the study are to be documented as AEs on the eCRF and as SAEs if case seriousness criteria are met),
    - medications, including vaccines, and other relevant treatments (e.g. surgeries) during 28 days prior to signing informed consent; all applicable treatments (systemic and local), also before 28 days prior to informed consent signing, for hematology/oncology disease(s) will be collected,
    - concomitant medications/treatments,
- measurement/assessment of
    - height and weight,
    - ECOG performance status (see Section 12.1),
    - vital signs including temperature, blood pressure (BP), and heart rate (HR) (BP and HR will be measured according to local standard practice);

[redacted]

- major body systems via physical examination; [redacted]

- safety blood samples for hematology, coagulation, serum chemistry, urinalysis, hormones, and virus serology to be performed and evaluated locally, [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
- [redacted]
- [redacted]

[redacted]

- a computer tomography (with contrast) of chest, abdomen, and pelvis will be performed at screening [redacted] if local SOC includes MRI instead of CT, this is also acceptable, but scanning type should, if at all possible, be consistent for an individual patient,
- a 12-lead electrocardiogram (ECG) will be recorded according to local site standard procedures, and
- adverse events will be reported according to NCI-CTCAE v5.0; documentation of AEs/SAEs starts after initial trial consent has been given by the patient.

### 7.4 Visits during study treatment

Visits V1 until V10 are planned to be performed as outlined in **Table 8**, including visit windows of +/- 2 days if nothing else stated in **Table 8** regarding specific items.

Study treatments are to be administered as outlined in Section 4.3, Section 6.3, and Section 6.4, and **Table 8**.

Measurements/assessments/sampling as outlined below are to be done before administration of study treatments if scheduled for the same day, if no other specific order of events is defined.

At all planned visits for treatments during the treatment period (see **Table 8**), the following items should be assessed and documented:

- concomitant medications/treatments,
- weight, and ECOG performance status (see Section 12.1),
- vital signs including temperature, blood pressure (BP), and heart rate (HR) (BP and HR will be measured according to local standard practice); [redacted]
- major body systems via physical examination; [redacted]
- safety blood samples for hematology, coagulation, serum chemistry, and urinalysis, to be performed and evaluated locally (as detailed in Section 7.3), and
- adverse events according to NCI-CTCAE v5.0.

The following items should be assessed/done and documented at the visits specified below (see also **Table 8**):

- [redacted]

[redacted]

### 7.5 Post-treatment safety visits

Thirty days (-0/+4 days) after the last administration of trial treatment, a safety visit [redacted] should be done for all patients, [redacted] The visits should if possible be held as planned, but there are patient individual circumstances that might prevent a personal visit to the site, e.g. the patient might be in medical care at another hospital and in a medical status not amenable to transportation. In such cases, the Investigator should try collecting as much of the normally collected information as possible from the sources available.

The items to be assessed/collected at visit [redacted] are (see also **Table 8**):

- concomitant medications/treatments,
- weight, and ECOG performance status (see Section 12.1),
- vital signs including temperature, blood pressure (BP), and heart rate (HR) (BP and HR will be measured according to local standard practice); [redacted]
- major body systems via physical examination; [redacted]
- safety blood samples [redacted]
- a 12-lead electrocardiogram (ECG) will be recorded according to local site standard procedures,
- for women of childbearing potential, a highly sensitive serum pregnancy test should be performed, and
- adverse events according to NCI-CTCAE v5.0.

### 7.6 Visits during post-treatment follow-up

#### *7.6.1 Post-treatment visits before determination of relapsed disease*

Patients who have permanently stopped all study treatment without having had confirmation of relapsed disease will be followed [redacted]

At these timepoints the following is to be done (see also **Table 8**):

- [redacted] CT scanning is to be done every 3-6 months per standard of care for the patient's stage of CRC disease (See Section 1.1 regarding recommendations for surveillance after curative treatments); collection of information from a protocol perspective should be done until scan documented relapse of disease (or until other

anti-cancer treatment would be started before confirmed progressive disease), or for a maximum of 3 years from first study treatment in the individual patient,

- [redacted] continue ctDNA testing per local standard of care; collection of information from a protocol perspective should be done until scan documented relapse of disease (or until other anti-cancer treatment would be started before confirmed progressive disease), or for a maximum of 3 years from first study treatment,
- [redacted]
- [redacted]
- [redacted]
- documentation of safety; [redacted]
- if applicable, for female patients of childbearing potential, a serum pregnancy test will continue to be done on a monthly basis until 6 months after study treatments have been stopped, if not a new anti-tumor treatment has been initiated earlier in which case the study related testing will be terminated at the time of start of new anti-tumor treatment, and
- if applicable, collection of information regarding further anti-cancer treatments.

[redacted]

### *7.6.2 Post-treatment visits after determination of relapsed disease*

Patients who have permanently stopped all study treatment and have had confirmation of relapsed disease will be followed [redacted]

If the patient is unable to attend a site visit, follow-up via a telephone contact is acceptable.

At these timepoints the following is to be done (see also **Table 8**):

- assessment of survival status [redacted]
- documentation of safety; [redacted]
- if applicable, for female patients of childbearing potential, a serum pregnancy test will continue to be done on a monthly basis until 6 months after study treatments have been stopped, if not a new anti-tumor treatment has been initiated earlier in which case the study related testing will be terminated at the time of start of new anti-tumor treatment, and
- collection of information regarding further anti-cancer treatments.

### 7.7 Safety assessments

Safety and tolerability of EO2040 in combination with nivolumab will be assessed by incidences of AEs, TEAEs, SAEs, deaths, reasons for treatment discontinuation/delays, and laboratory abnormalities. Adverse events will be categorized by their system organ class (SOC) and preferred term (PT) using the current MedDRA version and graded according to NCI-CTCAE v5.0 [100].

The trial safety setup is described in Section 4.3, the specific safety parameters to be monitored/assessed are outlined in Section 7, and safety monitoring procedures of the trial are described in Section 8. The timing of assessments is also described in **Table 8**.

Clinical laboratory analyses as described in **Section 7** will be performed by a local laboratory. More frequent evaluations may be performed at the Investigator's discretion if medically indicated; results of such evaluations should also be recorded in the eCRF. Normal ranges for all utilized local laboratories should be provided by the sites to the Sponsor.

### 7.8 Efficacy assessments

Response to therapy will be measured with a standard-of-care, CLIA certified ctDNA assay at the evaluation times specified in **Table 8**.

Identification of somatic mutations in the blood will be confirmed by sequencing of the resected tumor using a CLIA-certified next-generation sequencing panel. 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.

The primary endpoints will enumerate patients who have clearance of ctDNA, AND do not have any radiographic evidence of recurrence at 6 months.

Secondary endpoints include assessments of tumor recurrence (the word relapse is used interchangeably with recurrence) via scanning (preferably CT, but MRI is accepted if local SOC; in any case method to be used as consistently as possible in an individual patient throughout the study) or other evaluations such as endoscopy.

The best overall response is the best response recorded from the start of the treatment until disease recurrence. The patient's best response assignment will depend on the achievement of both ctDNA and imaging criteria (see **Table 9**).

[redacted]

## 7.9 Primary immunological assessments

### *7.9.1 Cell mediated immunity* [redacted]

Cell-mediated cytotoxicity is a key element in the proposed mechanism of action of the peptides composing EO2040. [redacted] Selection of an ex-vivo monitoring technique that provides a good measure of immune reactivity is important in determining potential correlations between clinical and immunologic responsiveness to specific immunotherapy.

Cellular immune response in this trial will be evaluated [redacted] Samples will be collected at screening and during the trial according to **Table 8**.

An important exploratory endpoint of the trial is the assessment of the percentage of patients with shown immunogenicity (expansion of specific T cells comparing samples taken at baseline versus on treatment in an individual patient determining if the patient has a positive response to the immunization, or not) in relation to the peptides that compose EO2040. Cross reactivities with the corresponding nominal peptides from BIRC5 and FOXM1 will also be evaluated.

As exploratory objectives of the trial, additional scientific information related to characterization of factors impacting antitumor immunity will be searched for with the intention to explore their influence on safety and/or efficacy parameters. [redacted]

Preparation/shipping procedures for blood samples will be detailed in the Laboratory Manual; a central laboratory will handle the cellular immune response analyses.

### *7.9.2 Correlations between immunogenicity and other trial outcome parameters*

As an exploratory objective, statistical assessments of correlations between immunogenicity [redacted] of the components of EO2040 [redacted], and efficacy and safety outcome parameters will be conducted. Cross reactivities shown for the nominal antigens BIRC5 and FOXM1 will also be explored in the same way.

## 7.10 Assessments for other exploratory endpoints

Exploratory endpoints are outlined in Section 3.2.3, beside what has already been described in Section 7.9, the following assessment types are going to be utilized:

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

For all parameters described above, when possible, the intention is to explore their influence on safety and efficacy outcome parameters of the patients in the trial.

# 8 SAFETY MONITORING

## 8.1 Safety monitoring definitions

### *8.1.1 Adverse events*

International Council for Harmonization (ICH) guideline E2A defines an AE as "any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment".

According to the FDA (21CFR312.32), an AE means any untoward medical occurrence associated with the use of a drug in humans, whether, or not, considered drug related.

*An AE can therefore be any unfavorable and unintended sign (e.g. tachycardia, enlarged liver), symptom (e.g. nausea, chest pain), abnormal result of an investigation (e.g. laboratory finding), or disease temporarily associated with the use of a medicinal product, whether or not considered related to the medicinal product.*

### *8.1.2 Serious Adverse events*

A serious adverse event (SAE) is any untoward medical occurrence that at any dose fulfills one or more of the following criteria:

- results in death,
- is immediately life-threatening,
    - *the term "life-threatening" in the definition of "serious" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe,*
- results in persistent or significant disability/incapacity,
    - *disability is defined as a substantial disruption in a person's ability to conduct normal life functions (i.e. the AE resulted in a significant, persistent, or permanent change, impairment, damage, or disruption in the patient's bodily function/structure, physical activities, or quality of life),*
- is a congenital anomaly/birth defect,
- requires inpatient hospitalization or prolongation of existing hospitalization, and
    - *a hospitalization is defined as an inpatient overnight stay, but this can be shorter than 24 hours,*
- is another medically significant event defined as an event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent any of the above listed outcomes.

#### *8.1.2.1 Excluded events*

Hospitalization for the following reasons will not be regarded as serious (i.e. will not be immediately reportable):

- routine treatment or monitoring of the disease under study, including hospitalization due to trial-related procedures, not associated with any deterioration of the patient's status,

- elective or pre-planned treatment (before signing the ICF) for a pre-existing condition that is unrelated to the disease under study and has not worsened since signing the ICF,
- treatment on an emergency outpatient basis for an event not fulfilling any of the definitions for an SAE and not resulting in hospital admission,
- underlying disease progression (the CRC which is the basis for the patient enrollment in the trial) and events which are unequivocally (without doubt) related to the disease progression, and
- social reasons, respite care, and in the absence of a medical condition.

In addition, as also outlined in Section 8.5.1, progression of the underlying disease or an AE unequivocally (without doubt) related to the progression of the underlying disease, regardless of its outcome or seriousness criteria, does not need to be reported as a SAE. However, the medical conditions and underlying diseases associated with disease progression needs to be captured on the AE eCRF page (as a non-serious AE). An SAE must be reported only if there are clinical symptoms/signs that cannot be without doubt associated with the progression of the underlying disease.

#### *8.1.3 Suspected Unexpected Serious Adverse Reactions (SUSARs)*

SUSARs are serious events that are not listed in the Reference Safety Information section of the EO2040 Investigator Brochure and are related to the study drug. Suspected adverse reaction means any AE for which there is a reasonable possibility that the study drug caused the AE. For the purposes of Investigational New Drug safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the study drug and the AE.

#### *8.1.4 Severity/intensity versus seriousness*

ICH E2A: The term "severe" is often used to describe the intensity (severity) of a specific event (as mild, moderate, or severe myocardial infarction); the event itself, however, may be of a relatively minor medical significance (such as a severe headache). This is not the same as "serious", which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

### 8.2 Pregnancies

If not surgically sterile, female patients of childbearing potential age must use highly effective contraception from signing the ICF through 6 months after the last treatment dose administered (see Section 5.1).

If not surgically sterile, male with female partner of childbearing potential age must use condom from signing the ICF through 6 months after the last treatment dose administered. Males must also ensure that their partners of childbearing potential use highly effective contraception (see Section 5.1).

Pregnancy is not considered an AE unless it meets any criteria for becoming serious (see Section 8.1.2). However, it is the responsibility of the Investigator or their designee to report immediately any pregnancy in a patient or the patient's sexual partner that occurs during the

study (see Section 8.5.3 regarding reporting procures). All patients who become pregnant must immediately discontinue the study drug and be withdrawn from study treatment.

The Investigator should make every effort to follow the patient until completion of the pregnancy and provide the corresponding information by the mean of a SAE/Pregnancy form within 2 weeks from awareness (see Section 8.5.3). The Sponsor Safety Group will periodically request from the investigator targeted follow-up information using a specific Pregnancy Follow-up form. If the events during pregnancy and/or outcome of pregnancy (i.e. complications regarding mother/baby) meet the criteria for classification of an SAE, the Investigator must follow the procedures for SAE reporting.

## 8.3 Safety monitoring periods

### *8.3.1 Reporting*

In patients exposed to study treatment, the following must be reported from the date of the patient's signing the ICF until 30 days after the final administration of study treatment:

- AEs regardless of their causal relationship to study treatment,
- SAEs without a plausible causal relationship to study treatment, and
- pregnancy of the patient or the patient's sexual partner.

[redacted]

SAEs with a plausible causal relationship to study treatment must be reported from the date of the patient's signing the ICF until indefinitely (regardless of the time elapsed from the final study treatment administration).

In patients not exposed to study treatment, the reporting period for AEs (including SAEs regardless of causal relationship) starts with signature of the ICF and ends:

- the date of the Investigators assessment of "screen failure"-status (see Section 5.3.2), or
- the date of the patient's consent withdrawal.

### *8.3.2 Follow-up*

The Investigator assesses at each visit (or more frequently, if necessary) if there are any new AEs or any changes in AE diagnosis, severity, suspected causal relationship to clinical trial medication/procedure, interventions required to treat the event, and AE outcome.

An AE causally not related to study treatment is monitored (followed-up) until resolution, stabilization (becoming a permanent condition), or end of the clinical trial. Clinically relevant laboratory abnormalities will be followed up until they return to normal or become stabilized (permanent condition).

Any AE with a plausible causal relationship to study treatment as well as all SAEs (regardless of their causal relationship) will be followed up until the event has resolved or stabilized (permanent condition).

Pregnancies will be monitored by the Investigator to determine the outcome, including spontaneous abortion or voluntary termination, birth details, and the presence or absence of any birth defects, congenital abnormalities, or maternal and newborn complications. Every infant should be followed up for 2 months after delivery.

## 8.4 Assessing adverse events

Information about adverse reactions (causally related events) already known for EO2040 monotherapy, or EO2040 in combination with nivolumab, can be found in the current version of the EO2040 IB or will be communicated between IB updates in the form of "Dear-Investigator Letter".

Information about adverse reactions (causally related events) already known for nivolumab can be found in the labelling documents for nivolumab [90, 91].

### *8.4.1 Causality*

The Investigator needs to assess the causal relationship of any AE in relation to the study treatments. In context of the study treatments, EO2040 and nivolumab are given in combination in Cohort 1, it is most of the times in principle not possible to make causality assessments for each component by itself.

However, there will be a possibility in the eCRF to record causality for EO2040 and nivolumab as separate components for each AE, or to select to make an overall assessment for both study treatments together.

The causality assessment is based on the Investigator's clinical judgment taking into consideration all relevant information available at the time of AE reporting including:

- temporal association of the event onset with administration of the medication,
- known type of reaction for the administered compounds,
- disappearance or abating of symptoms when the compound(s) is discontinued, or withheld,
- reappearance of symptoms when the compound is re-administered,
- event may or may not be caused by the patient's health condition,
- presence of risks or factors not related to trial intervention that are known to be associated with the occurrence of the event.

Causal relationship of all AEs will be classified as follows:

- **Not suspected**: it is not plausible that the AE is caused by the medication(s) and a likely alternative explanation exists. *There is no reasonable possibility of a causal relationship.*
- **Suspected**: it is plausible that the AE is caused by the medication(s). *There is a reasonable possibility of a causal relationship.*

  *For the purposes of safety reporting,* ***'reasonable possibility'*** *means there is evidence to suggest a causal relationship between the medication(s) and the AE.*

### *8.4.2 Severity/intensity*

Severity or intensity of an AE should be assessed according to the NCI CTCAE v5.0 [100]; a grading scale is provided for AE terms displaying grades 1 through 5 with unique clinical descriptions of severity for each AE. Note, possible changes of the severity/intensity of adverse events should be monitored, and when applicable documented.

## 8.5 Reporting by the investigational site

### *8.5.1 Adverse events*

Any AE (including SAEs), whether, or not considered to be causally related to the trial medication and regardless of its seriousness, must be described and recorded in the AE section of the patient's eCRF on an ongoing basis.

A pre-existing condition is a clinical condition (including a condition being treated) that is diagnosed before the patient signs the ICF and that is documented as part of the patient's medical history; any clinically significant worsening of a pre-existing condition constitutes an AE. Also, any recurrence of a pre-existing condition is an AE.

The questions concerning whether the condition existed before the start of the active phase of the study and whether it has increased in severity and/or frequency will be used to determine whether an event is a TEAE. An AE is considered to be treatment-emergent if it is not present when the active phase of the study begins and is not a chronic condition that is part of the patient's medical history, or it is present at the start of the active phase of the study or as part of the patient's medical history, but the severity or frequency increases during the active phase. The active phase of the study begins at the time of the first dose of the study drug. The active phase of the study ends at the post-treatment visit for safety assessment (see **Table 8**).

A standardized question such as "Have you had any health problems since your last visit or since you were last questioned?" will be given by the Investigator or the investigational site personnel at each contact with the patient.

Progression of the underlying disease or an AE unequivocally (without doubt) related to the progression of the underlying disease, regardless of its outcome or seriousness criteria, does not need to be reported as an AE/SAE. However, the medical conditions and underlying diseases associated with disease progression needs to be captured on the AE eCRF page (as a non-serious AE). An SAE must be reported only if there are clinical symptoms/signs that cannot be without doubt associated with the progression of the underlying disease.

Whenever possible, a diagnosis rather than symptoms should be provided (e.g. anemia instead of low Hb).

Abnormal laboratory and other abnormal examinations (e.g. at physical examination) should not be reported as AEs, unless they are associated with clinical signs/symptoms, require medical intervention/therapy (e.g. transfusion due to low Hb), require a change in trial medication (e.g. temporary interruption of treatment, or definitive treatment discontinuation), or are otherwise considered clinically relevant by the Investigator. Clinically relevant laboratory, or examination abnormalities will be followed up until they return to normal or become stabilized (permanent condition).

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g. ALP and bilirubin 5 times the ULN associated with cholecystitis), only the diagnosis (i.e. cholecystitis) should be recorded on the AE eCRF page.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the AE eCRF page, along with a descriptor indicating if the test result is above or below the normal range (e.g. "elevated potassium," as opposed to "abnormal potassium"). If the laboratory abnormality can be characterized by a precise clinical term per standard definitions, the clinical term should be recorded as the AE.

For example, an elevated serum potassium level of 7.0 mEq/L should be recorded as "hyperkalemia."

Physical examination findings will be compared with the baseline status and any clinically significant change, as assessed by the Investigator, should be documented as an AE.

A surgical procedure is not an AE but a therapeutic measure for a condition that necessitates surgery. Therefore, the condition for which the surgery is required should be reported as an AE.

Any pre-planned surgery (i.e. planned before signature of the ICF) or other intervention permitted by the protocol and the condition leading to that measure are not AEs. In such cases, the underlying condition needs to be documented in the patient's medical history.

Death itself is an outcome of an event, which needs to be described and reported using medical terminology. Information about death will be captured on the respective eCRF page along with relevant details (date of death, immediate and underlying causes of death).

#### *8.5.1.1 Adverse Events Associated with an Overdose or Error in Treatment Administration*

Study drug overdose is the accidental, or intentional, use of the drug in an amount higher than the dose being studied. An overdose, or incorrect administration, of study drug is not an AE unless it results in untoward medical effects.

Any study drug overdose or incorrect administration of study drug should be noted on the Study Drug Administration eCRF page.

All AEs associated with an overdose or incorrect administration of study drug should be recorded on the AE eCRF page. If the associated AE fulfils serious criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 8.5.3 for reporting instructions).

### *8.5.2 Documenting in the eCRF*

The reported term should be a medical diagnosis or sign/symptom of the event, not a procedure. Each symptom in a constellation of symptoms should be listed separately if the Investigator has not made a preliminary/tentative summary diagnosis.

Fluctuations or re-occurrences of a condition, which are considered normal for the patient and are recorded in patient's eCRF medical history, do not need to be reported as an AE. However, if the condition deteriorates during the trial it needs to be captured as an AE.

If the same AE occurs repeatedly, it must be assessed and documented separately each time if there are "AE-free" time periods between the AEs.

If possible, each AE should be evaluated to determine:

- event term or a description of the AE in medical terms (not as reported by the patient),
- severity grade as assessed by the Investigator (1 - 5 per NCI CTCAE v5.0),
- its causal relationship to study treatment(s) as assessed by the Investigator (suspected; not suspected),
- event duration, including onset date and end date,
- action taken with study treatments due to the reported event (dose not changed; drug interrupted, drug withdrawn; other),

- other action taken (no action taken; medication required; surgical intervention required; other),
- event seriousness (non-serious or serious AE),
- event outcome (recovered/resolved, recovered/resolved with sequelae, not recovered/not resolved, fatal, unknown), and
- disease progression-related event (yes, no).

### *8.5.3 Immediately reportable events - Serious Adverse Events & Pregnancies*

The Investigator or designated investigational site staff must immediately (within 24 hours of awareness at the latest) notify/report to pharmacovigilance at the Sponsor, [redacted], any initial or medically relevant follow-up information about SAEs, or pregnancies (see Section 8.2).

All immediate reports from the investigational site to the Safety Group (i.e. SAEs and pregnancy reports) must also be recorded in the site's source documentation.

The primary mode of reporting a SAE is based on entering the appropriate and complete information into the clinical database via the SAE reporting form and submitting the SAE form [redacted] the trial Safety Group within 24 hours of obtaining knowledge of the event.

The SAE Form [redacted] is to collect data surrounding the event (e.g. the nature of the symptom[s], time of onset in relation to initiation of therapy, duration, intensity, and whether, or not therapy was interrupted or discontinued). The Investigator's assessment of the probable cause of the event will also be included. In addition, relevant medical history, concomitant medications, laboratory and diagnostic test reports, and procedures as well as all pertinent medical information related to the event will also be collected. [redacted] the site will submit it to the safety group within the system. Then an alert from the clinical database will be initiated and sent to the Safety Group. The Safety Group will generate the SAE report, a PDF extracted from the clinical database, and send to the Sponsor.

The Safety Group will generate SAE queries requesting missing information, correction of implausible, etc., and add the queries directly in the clinical database. It is the Investigator's responsibility to be diligent in providing the answer as soon as it is available by entry of the corrections in the eCRF and submission of the follow-up SAE report to the Safety Group.

[redacted]

*Note, SAE report forms must be completed in English.*

When a paper form is completed, follow-up information should not be reported on the same form that was used for the initial reporting, but using a new form filled in only with the new information. Changes/completions need to be done in a Good Clinical Practice (GCP)-compliant manner (i.e. dated and initialized). Originals of the report forms must be kept in the site study file (if certified electronic signature is available at site, electronic copy of the form is acceptable).

Initial reports of SAEs should never be left on telephone voicemails. In case of using the paper SAE form, please always fax or e-mail the SAE report, and follow-up with a telephone call if needed.

**Serious AEs to be reported to the Enterome/**[redacted]

[redacted]

**In case of urgent questions regarding SAE reporting please call:**

[redacted]

The initial Pregnancy Data Collection form should be handled in the same way as an initial SAE report, i.e. via the EDC system, or via the specific paper form (latter only in case of technical issues with the EDC).

Follow-up information is to be sent within the same timelines using the same modes of reporting as outlined above.

All sites will follow their institutional requirements for submission of SAEs to their IRBs/IECs.

The processing and reporting of all relevant SAEs to authorities will be done by the Sponsor or Sponsor's designee according to all applicable rules/regulations, including reporting of unexpected serious adverse events to the USA FDA according to 21 CFR 312.32. The Sponsor or designee will inform all investigational sites about reported relevant events according to applicable regulations.

[redacted]

#### *8.5.3.1 SAE minimum notification/reporting requirements*

The following information must be provided for a valid notification/report:

- identification of the notifying/reporting person (e.g. name of the reporter),
- identification of the patient (e.g. patient trial identification number),
- concerned IMP and/or clinical trial (e.g. EO2040, trial EOCRC1-22),
- reason for notification/reporting (i.e. SAE, or pregnancy), and
- event term.

*In addition, providing the **assessment of the causal relationship** is necessary for comprehensive evaluation by the Sponsor.*

### 8.6 Independent Data Monitoring Committee (IDMC)

The IDMC will serve as an external monitoring group for the study. The primary role of the IDMC will be to examine the safety and tolerability of study participants throughout the duration of the study. The IDMC will be created to further protect the rights, safety, and well-being of patients who will be participating in the trial by monitoring their progress and results.

[redacted]

The IDMC members, including a minimum of three qualified persons, are to be international hematology/oncology experts, or qualified clinical trial statistician (there is no demand for statistical expertise among the IDMC members for this trial), who are not in other ways than via the IDMC involved in the current trial. Details of the IDMC processes and procedures is outlined in a separate IDMC Charter.

# 9 STATISTICAL EVALUATION

## 9.1 Analysis populations

The All-Patient (AP) population 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]

Efficacy will be analyzed using both the FAS and PP populations. Safety will be analyzed using the SS, however applicable adverse events for the AP population will be reported in by-patient listings (e.g. events during screening).

## 9.2 Statistical methods

All data collected in this trial will be reported by patient data listings, summary tables, and figures for all demographic and baseline characteristics, medical history, efficacy, and safety variables. Proportions and the denominators will be provided to summarize response, toxicity, and other categorical variables.

[redacted]

Statistical assessments of correlations between e.g. immunogenicity parameters (see Section 3.2.3, and Section 7.9) and efficacy and safety outcome parameters will be conducted as described in the Statistical Analysis Plan (SAP).

The SAP will provide all details with respect to patients being analyzed for all endpoints. Relevant subgroups based on available data and compliance to protocol procedures might be defined.

Statistical tests may be planned in the SAP or performed as needed. The importance of the tests does not reside in making conclusions on statistical relevance of differences, but rather identification of possible results that could be worth further exploration.

## 9.3 Interim analyses

The nature of the study, i.e. early exploratory development trial aiming at generating as much knowledge as possible before potential decisions related to further development of EO2040, makes it important to assess especially safety, but also efficacy and possible biomarkers, on an ongoing basis during trial conduct. Exploratory preliminary data from the trial might be utilized in relation to e.g. scientific discussions and presentations to facilitate input regarding possible improvements of development parameters.

[redacted]

### 9.4 Determination of sample size & primary analysis

This is an early development, open-label, exploratory, trial to include patients with CRC who have early disease which today is without an established standard of care, but still in a relevantly early stage of the overall disease history that there is a potential chance to significantly delay recurrence/progression with an efficacious therapy. Thus, a balance needs to be struck between a size to find a relevant signal, and the importance to not expose too many patients to a possibly non-efficacious therapy.

Considering prior attempts in similar situations, the trial will include 17 patients per cohort.

[redacted]

The primary objective of the trial is to determine the ctDNA clearance rate at 6 months (R6).

[redacted]

For the primary analysis, the ctDNA clearance rate will be estimated along with the 95% exact confidence interval. The efficacy of study treatment will also be assessed by performing a binomial test comparing the ctDNA clearance rate against the null hypothesis 5%, at a one-sided alpha level of 0.025.

[redacted]

The opening of a Cohort 2A exploring EO2040 monotherapy (with the option of addition of nivolumab if ctDNA is not cleared at 3 months) is dependent on the outcome of Cohort 1; [redacted] If no continuation of exploration of EO2040 would be seen as appropriate based on data from Cohort 1, there will be considerations regarding a protocol amendment to switch EO2040 to an alternative microbiome-derived vaccine.

Based on the above, it is assumed that the current trial will include enough patients to provide safety and tolerability information, immunogenicity data, as well as preliminary efficacy

data in the selected treatment setting without demanding a too high (and long) patient recruitment and without exposing too many patients.

# 10 ADMINISTRATIVE CONSIDERATIONS

## 10.1 Regulatory and ethical considerations

This study will be conducted in accordance with the protocol and with the following:

- consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines,
- applicable International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human Use (ICH) Good Clinical Practice (GCP) Guidelines, and
- applicable laws and regulations; approval will be obtained from the appropriate regulatory authorities before any site is initiated in a country.

Before study start, each site Principal Investigator (PI) is required to sign a protocol signature page confirming his agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to clinical research associates (CRAs), auditors, the Sponsors quality assurance representatives, designated agents of the Sponsor, ECs, and regulatory authorities as required.

The protocol, protocol amendments, Informed Consent Form (ICF), Investigator's Brochure, and other relevant documents (e.g. advertisements) must be submitted to an Institutional Review Board (IRB)/Independent Ethics Committee (IEC) by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.

The Investigator will be responsible for the following:

- providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC,
- notifying the IRB/IEC of serious adverse events (SAEs) or other significant safety findings as required by IRB/IEC procedures, and
- providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.

A signed and dated statement that the protocol and ICF have been approved by the ECs must be given to the Sponsor (or designee) before study initiation.

## 10.2 Finances and insurances

Financing and insurance will be addressed in separate site agreements.

For each participating patient, the Sponsor has taken out insurance covering the amount determined by respective national laws. All participating patients will be informed about the existence of the insurance in the patient informed consent. They have the right to review the terms and conditions of the insurance.

### 10.3 Informed consent

Written informed consent for the study will be obtained from all patients before any protocol-specific procedures are conducted. The ICF generated by the Sponsor or designee will be approved (along with the protocol) by the IRB/IEC.

Information about the study will be given to the patient both verbally and in writing. The written patient information sheet will explain the objectives of the study and its potential risk and benefits. The patient should have adequate time to read the information sheet and to ask the Investigator any questions. The Investigator must be satisfied that the patient has understood the information provided before written consent is obtained. If there is any doubt as to whether the patient has understood the written and verbal information, the patient should not enter the study.

If a patient agrees to participate, he/she will be asked to sign and date the study ICF, which will be retained by the Investigator. A copy of the signed ICF will be given to the patient. The informed consent process must be documented in the patient's source documents. The original ICF must be retained by the Investigator and made available for inspection by the Study Monitor.

### 10.4 Future use of patient samples; sample traceability

[redacted]

Blood samples being collected and stored for research purposes within this study should be traceable as required by local regulations, or the clinical trial authorization, as applicable. The Sponsor and Investigator institutions should keep their parts of the traceability records accordingly.

### 10.5 Patient data protection

Patients will be assigned a unique identifier and will not be identified by name in eCRFs, study-related forms, study reports, or any related publications. Patient and Investigator personal data will be treated in compliance with all applicable laws and regulations. In the event the study protocol, study report, or study data are included in a public registry, all identifiable information from individual patients or Investigators will be redacted according to applicable laws and regulations.

The patient must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the patient. The patient must also be informed that his/her medical records may be examined by the Sponsor or Contract Research Organization (CRO) auditors, or other authorized personnel appointed by the Sponsor, and by inspectors from regulatory authorities.

### 10.6 Site monitoring

Before study initiation, at a site initiation visit or at an Investigator's meeting, Sponsor personnel (or a designated contract research organization [CRO]) will review the protocol and eCRFs with the Investigators and their staff. During the study, the field monitor will visit the site regularly to check the completeness of patient records, the accuracy of filling of the eCRFs, the adherence to the protocol and to GCP, the progress of enrollment, and to ensure that the study drug is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits.

The Investigator must maintain source documents for each patient in the study recruited at the relevant site, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, ECGs, and the results of any other tests or assessments. All information on eCRFs must be traceable to these source documents in the patient's file. The Investigator must also keep the original ICF signed by the patient (a signed copy is given to the patient).

The Investigator must give the monitor access to all relevant source documents to confirm their consistency with the eCRF filling. The Sponsor monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and the recording of data that will be used for all primary and safety variables. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific monitoring plan. No information in source documents about the identity of the patients will be disclosed.

### 10.7 Data collection

All individual, patient-specific study data will be entered into a 21 CFR Part 11-compliant electronic data capture (EDC) system on an eCRF in a timely fashion.

The Sponsor, or designee, will supply the investigational site with access to a web-based EDC computer system. The designated Investigator site staff will not be given access to the EDC system until they have been trained.

Access to the EDC system at the site, for vendors, at the Sponsor, and at the CRO is password protected. Study access is granted to site personnel only after they have been trained in the use of the EDC system by web-based training at the investigational site.

The EDC system contains a system generated audit trial that captures any changes made to a data field, including who made the change, and the date and time it was made. This information is available at the Investigator's site, and at the Sponsor (and CRO when applicable).

Data will be entered into the study database by the Investigator/Study Coordinator at each site. [redacted]

Data generated from external sources (e.g. central laboratory) and transmitted to the Sponsor or designee electronically might be integrated with the patient's eCRF data in accordance with the Data Management Plan.

An eCRF must be completed for each patient who signs an ICF and undergoes any pre-screening or screening procedures, according to the eCRF completion instructions.

Automatic validation programs check for data discrepancies in the eCRFs and, by generating appropriate error messages, allow modification or verification of the entered data by the Investigator staff before transfer of data to the Sponsor (or a designated CRO).

Sponsor personnel (or a designated CRO) will review the eCRFs entered by site staff for completeness and accuracy (including as applicable verification against source documents) and instruct the site personnel to make any required corrections or additions. System or manually generated queries are raised to the investigational site using the study EDC system. Designated Investigator/site staff is required to respond to the queries and make any necessary changes to the data.

The Investigator will sign and date the eCRF via the EDC system's electronic signature procedure. These signatures will indicate that the Investigator reviewed and approved the data on the eCRF, the data queries, and the site notifications.

At the conclusion of the study, the occurrence of any protocol violations will be determined (guidance can be found in Section 12.3).

After these actions have been completed and the database has been declared to be complete and accurate, it will be locked.

After database lock, the Investigator will receive a CD-ROM of the patient data for archiving at the investigational site.

### 10.8 Protocol amendments

Any substantial amendments in the research protocol during the period, for which the IEC/IRB approval had already been given, will not be initiated without submission of an amendment for IEC/IRB review and approval.

These requirements for approval will in no way prevent any immediate action from being taken by the Investigator in the interest of preserving the safety of all patients included in the trial.

### 10.9 Protocol deviations and violations

A protocol deviation is any departure from the defined procedures and treatment plans as outlined in the protocol version submitted and previously approved by the IEC/IRB. Protocol deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study, thus jeopardizing the justification for the research. Protocol deviations are unplanned and unintentional events.

Important protocol deviations, such as significant non-compliance or other serious unforeseen deviations deemed to invalidate the data collected in lieu of the purpose of the study will lead to exclusion of data from analysis.

All decisions regarding the type of deviations will be made prior to commencing the final analysis on the finally locked database. A listing of patients with protocol deviations will be maintained by the Sponsor (or designee) and a listing of protocol violations (see Section 12.3) will be presented in the final study report.

Investigators shall apply due diligence to avoid protocol deviations. If the Investigator feels a protocol deviation would improve the conduct of the study, this must be considered a protocol amendment, and unless such an amendment is agreed upon by the Sponsor and approved by the EC and concerned regulatory authorities, it cannot be implemented.

The Investigator will report protocol deviations to their IEC/IRB per institutional reporting requirements.

### 10.10 Change in investigator

If any Investigator retires, relocates, or otherwise withdraws from conducting the study, the responsibility for maintaining records may be transferred to the Sponsor or designee, IRB, or another Investigator. The Sponsor or designee must be notified of and agree to the change. Regulatory agencies will be notified with the appropriate documentation.

### 10.11 Clinical study report

A clinical study report will be prepared following the completion of the study. The Global Coordinating Investigator should if required by local law/regulations be the Investigator who should review and sign the study report.

### 10.12 Confidentiality/disclosure

All information provided regarding the study, as well as all information collected and/or documented during the study, will be regarded as confidential. The Investigator (or designee) agrees not to disclose such information in any way without prior written permission from the Sponsor.

### 10.13 Record retention

Essential documents must be retained for longer than 5 years after completion of the study, 2 years after the final marketing authorization in an ICH region or until at least 2 years have elapsed since the discontinuation of clinical development of the study drug. If it becomes necessary for the Sponsor or a relevant competent authority to review any documentation relating to the study, the Investigator must permit access to such records.

Study files may be discarded upon written notification by the Sponsor. To avoid error, the Investigator must contact the Sponsor before destroying any records or reports pertaining to the study, to ensure that retention is no longer required. Other source documents, such as patient's medical records, must be retained for the maximum time permitted by the hospital or institution and until such time when the Investigator is informed by the Sponsor that there is no further need to do so.

In addition, in accordance with the Investigator agreement, the Sponsor should be contacted if the site Principal Investigator plans to leave the investigational site so that appropriate arrangements can be made.

### 10.14 Publications

The main data generated in trial EOCRC1-22 will be published, whether positive or negative, that the knowledge generated by performing the trial will become public (including the administrative information regarding the trial, including authority/ethic committee approvals). Timing of the publication of data will be driven by the Global Coordinating Investigator. When based on preliminary data, or on completion of the study, the data warrant publication/presentation according to a judgement by the Global Coordinating Investigator after collaborative discussions with other involved Investigators (meaning such Investigators who have recruited patients to the trial or have had a significant participation in the trial by other means), the results may be published/presented, under the auspice of the Global Coordinating Investigator, in a recognized scientific journal, or at a scientific conference, subject to the provisions of the following process:

- No publication based on the results obtained at an individual trial site (or group of sites) shall be made before the first multicenter publication or presentation unless otherwise agreed in writing. Notwithstanding the foregoing, if a multicenter publication is not published within twelve (12) months after completion of the clinical trial and final lock of the clinical trial database at all research sites that are part of the multicenter clinical trial, or any earlier termination or abandonment of the clinical trial, or if the Sponsor informs the Principal Investigator that such multicenter publication will not take place, or if publication has been agreed otherwise, the site Principal Investigator shall have the right to publish or present the methods and results obtained at his/her site of the clinical trial.
- In case a publication/presentation is proposed, the Global Coordinating Investigator shall discuss the proposed publication/presentation with the Sponsor and give the Sponsor a relevant time (depending on the planned publication situation; e.g. referred journal, or scientific conference abstract, submission) to support with data collection and analyses which could be the basis for a publication.
- The Sponsor should have adequate time for review of proposed abstracts, manuscripts, and/or other presentation materials before submissions (time is dependent on the planned publication situation; adequate time for review before submission of a complete manuscripts to a referred journal can be considered as 30 days, to respond with any requested revisions, including the deletion of confidential information which must be done).

# 11 LITERATURE

## 11.1 References

1. [redacted]

18. [redacted]

38. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

# 12 APPENDICES

## 12.1 Appendix 1: ECOG performance status

The performance status grading as outlined below was developed by the Eastern Cooperative Oncology Group (ECOG) [101].

| GRADE | ECOG PERFORMANCE STATUS |
|---|---|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work |
| 2 | Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours |
| 3 | Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled; cannot carry on any selfcare; totally confined to bed or chair |
| 5 | Dead |

## 12.2 Appendix 2: New York Heart Association functional classification

The New York Heart Association (NYHA) functional classification is described below [102].

| **Class** | **Patient Symptoms** |
|---|---|
| Class I (None) | No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath). |
| Class II (Mild) | Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea. |
| Class III (Moderate) | Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea. |
| Class IV (Severe) | Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased. |

## 12.3 Appendix 3: National Institute of Health (NIH) deviation definitions

*Notes:*

- *the term "subject" has been switched to "patient" by the Sponsor in the below text, and*
- *in trial EOCRC1-22 the terminology only includes the word "Deviation", which is subdivided into "Major Deviations" (corresponding to the "Protocol Violation" term as defined by NIH below, when the definition for "Minor Deviation" is not fulfilled), and "Minor Deviations" (corresponding to "Minor Protocol Deviation" as defined by NIH below).*

**NIH Institutional Review Board Professional Administrators Committee Version 5.1**

**Regulatory Process Workgroup 11/18/2005**

[http://www.genome.gov/Pages/Research/Intramural/IRB/Deviation_Violation_examples8-07.pdf; accessed March 13, 2022] [103]

**Protocol Deviations and Violations**

**Protocol Deviation** - A protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that is under the Investigator's control and that has not been approved by the EC. Upon discovery, the Principal Investigator is responsible for reporting protocol deviations to the EC using the standard reporting form.

*Any change, divergence, or departure from the study design or procedures of a research protocol that affects the patient's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data constitutes a protocol violation.*

*Changes or alterations in the conduct of the trial which do not have a major impact on the patient's rights, safety or well-being, or the completeness, accuracy, and reliability of the study data are considered minor protocol deviations.*

**Protocol Violation** - A protocol violation is a deviation from the EC approved protocol that may affect the patient's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data.

*If the deviation meets any of the following criteria, it is considered a protocol violation.*

Example list is not exhaustive.

**I. The deviation has harmed or posed a significant or substantive risk of harm to the patient.**

Examples:

- a patient received the wrong treatment or incorrect dose,
- a patient met withdrawal criteria during the study but was not withdrawn, and
- a patient received an excluded concomitant medication.

**II. The deviation compromises the scientific integrity of the data collected for the study.**

Examples:

- a patient was enrolled but does not meet the protocol's eligibility criteria,
- failure to treat patient per protocol procedures that specifically relate to primary efficacy outcomes (if it involves patient safety it meets the first category above),
- changing the protocol without prior EC approval, and
- inadvertent loss of samples or data.

**III. The deviation is a willful or knowing breach of human patient protection regulations, policies, or procedures on the part of the Investigator(s).**

Examples:

- failure to obtain informed consent before initiation of study-related procedures,
- falsifying research or medical records, and
- performing tests or procedures beyond the individual's professional scope or privilege status (credentialing).

**IV. The deviation involves a serious or continuing noncompliance with federal, state, local, or institutional human patient protection regulations, policies, or procedures.**

Examples:

- working under an expired professional license or certification,
- failure to follow federal and/or local regulations, and intramural research or clinical center policies, and
- repeated minor deviations.

**V. The deviation is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles.**

Examples:

- a breach of confidentiality, and
- inadequate or improper informed consent procedure.

**Minor Protocol Deviation** is any change, divergence, or departure from the study design or procedures of a research protocol that has not been approved by the EC and which DOES NOT have a major impact on the patient's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

## 12.4 Appendix 4: Administration-related reactions

The NCI-CTCAE distinguishes between hypersensitivity reactions and acute infusion reactions induced by cytokine release. Despite the different possible mechanisms underlying hypersensitivity and infusion reactions, the clinical signs and symptoms associated with these reactions overlap.

Immediate systemic reactions, infusion-related reactions and hypersensitivity reactions, at administration of nivolumab can be considered to be common [90, 91]. Systemic reaction at administration of EO2040 cannot be excluded either, even if not reported in the early development of the similar compound EO2401(see Section 1.3.2).

*NOTE: At administration of the treatment compounds of this trial, immediate treatment of severe allergic reactions should be available, including staff well trained in resuscitation, IV access for administration of fluids, antihistamines, corticosteroids, and epinephrine (adrenaline).*

***Treatment of administration-related reactions***

If such reactions would occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypotension, hypertension, bronchospasm, or other allergic-like reactions. All Grade ≥ 3 infusion reactions should be reported within 24 hours to the Sponsor via the Medical Monitor and be reported as an SAE if it meets the criteria. Infusion reactions should be graded according to NCI-CTCAE v5.0.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines, as appropriate:

- For Grade 1 symptoms: (mild reaction; infusion interruption not indicated; intervention not indicated):
    - Remain at bedside and monitor patient until recovery from symptoms. The following prophylactic pre-medications are recommended for future infusions; diphenhydramine 50 mg (or equivalent), and/or acetaminophen 325 to 1000 mg orally at least 30 minutes before additional administrations.
- For Grade 2 symptoms: (moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g. antihistamines, nonsteroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for ≤ 24 hours):
    - Stop the infusion, begin an IV infusion of normal saline, and treat the patient with diphenhydramine 50 mg IV (or equivalent), and/or acetaminophen 325 to 1000 mg PO; remain at bedside and monitor patient until resolution of symptoms. Corticosteroid and/or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient closely. If symptoms recur, then no further drug will be administered at that visit.
    - For future infusions, the following prophylactic pre-medications are recommended: diphenhydramine 50 mg (or equivalent), and/or acetaminophen/paracetamol 325 to 1000 mg PO administered at least 30 minutes before infusions. If necessary, corticosteroids (up to 25 mg IV of hydrocortisone or equivalent) may be used.

- For Grade 3 or 4 symptoms: (severe reaction, Grade 3: prolonged [i.e. not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g. renal impairment, pulmonary infiltrates]. Grade 4: life-threatening; pressor or ventilatory support indicated):
    - Immediately discontinue infusion of the compound. Begin an IV infusion of normal saline and treat the patient as follows: recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10 000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. The patient should be monitored until the Investigator is comfortable that the symptoms will not recur. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor patient until recovery of the symptoms. Further treatment of the patient should be held, and assessments made per the general and specific safety rules of the trial.

***Late-occurring hypersensitivity symptoms***

In case of late-occurring hypersensitivity symptoms (e.g. appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g. oral antihistamine or corticosteroids).

## 12.5 Appendix 5: Management algorithms regarding nivolumab for studies under CTCAE version 5.0

***These general guidelines are standard nivolumab protocol safety algorithms (28-Sep-2020) and recommended for inclusion in any nivolumab containing protocol by Bristol-Myers Squibb (marketing authorization holder for nivolumab).***

These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the Medical Monitor representing the Sponsor. The guidance applies to all immuno-oncology agents and regimens.

A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated.

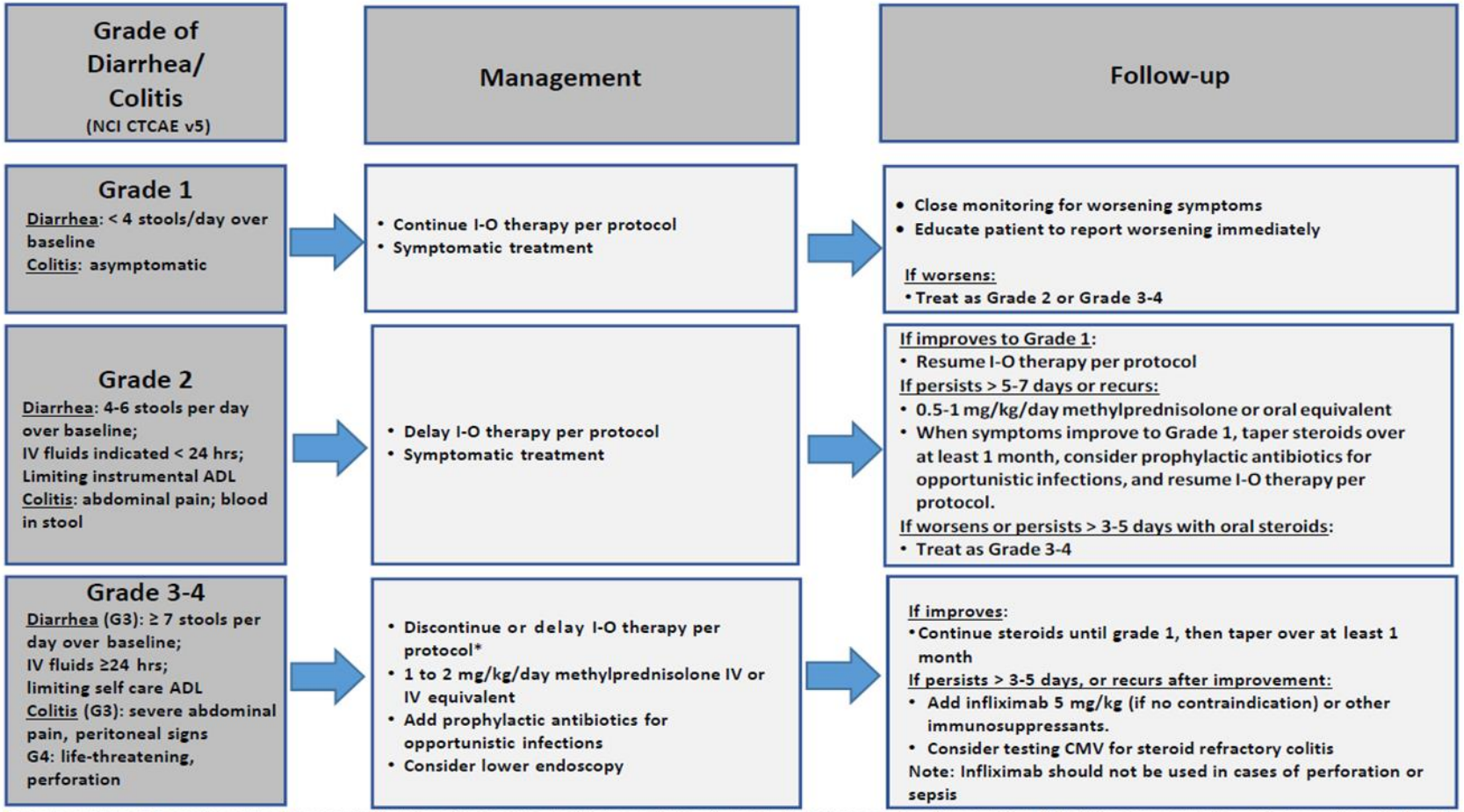
Corticosteroids are a primary therapy for immuno-oncology drug-related adverse events. The oral equivalent of the recommended IV doses may be considered for ambulatory patients with low-grade toxicity. The lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or procedure, is recommended.

The frequency and severity of the related adverse events covered by these algorithms will depend on the immuno-oncology agent or regimen being used.

# GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy.
Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

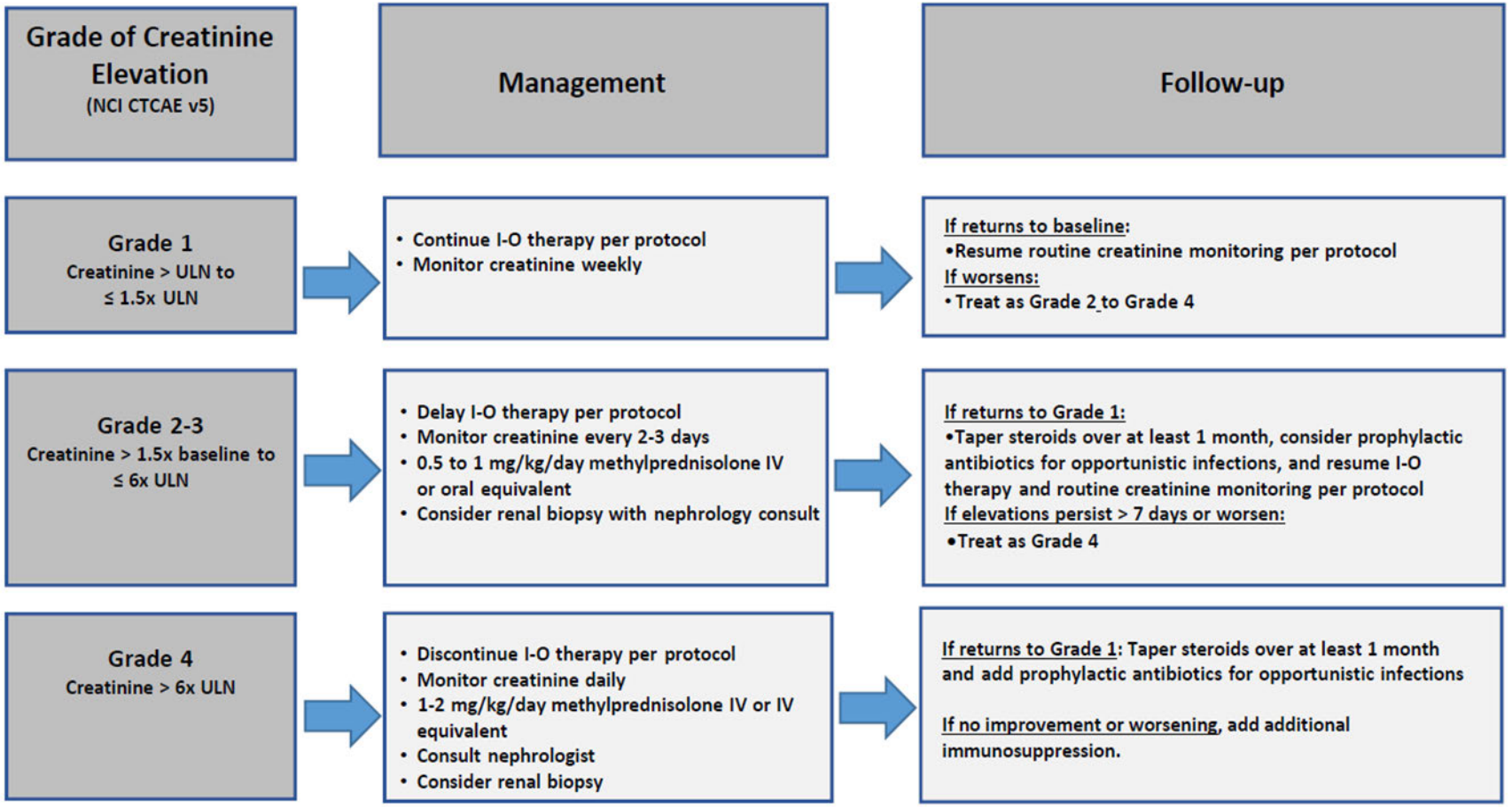



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

* Discontinue for Grade 4 diarrhea or colitis. For Grade 3 diarrhea or colitis, 1) Nivolumab monotherapy: Nivolumab can be delayed. 2) Nivolumab+ Ipilimumab combination: Ipilimumab should be discontinued while nivolumab can be delayed. Nivolumab monotherapy can be resumed when symptoms improve to Grade 1. Please refer to protocol for dose delay and discontinue criteria for other combinations.

# Renal Adverse Event Management Algorithm

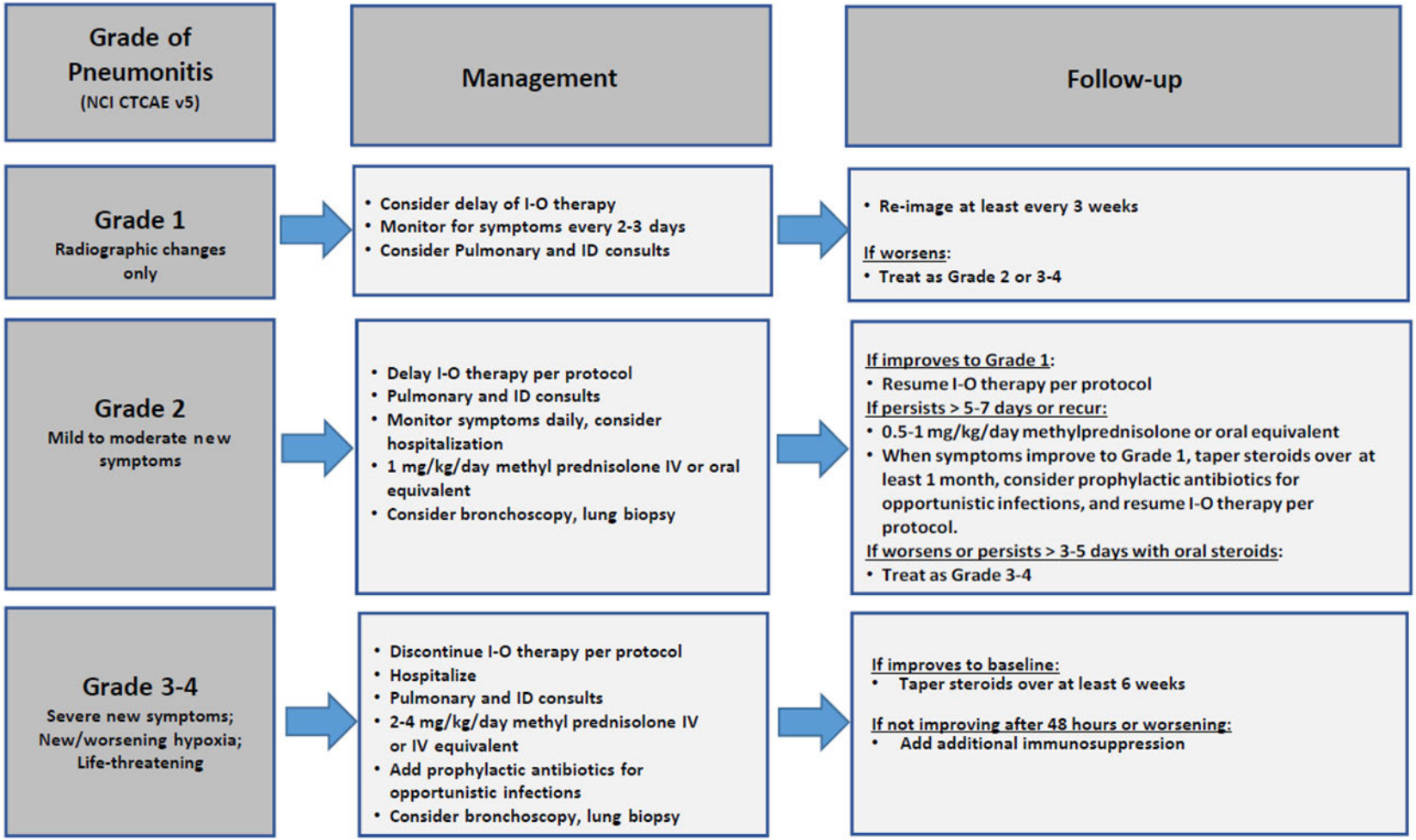
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.




Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

# Pulmonary Adverse Event Management Algorithm

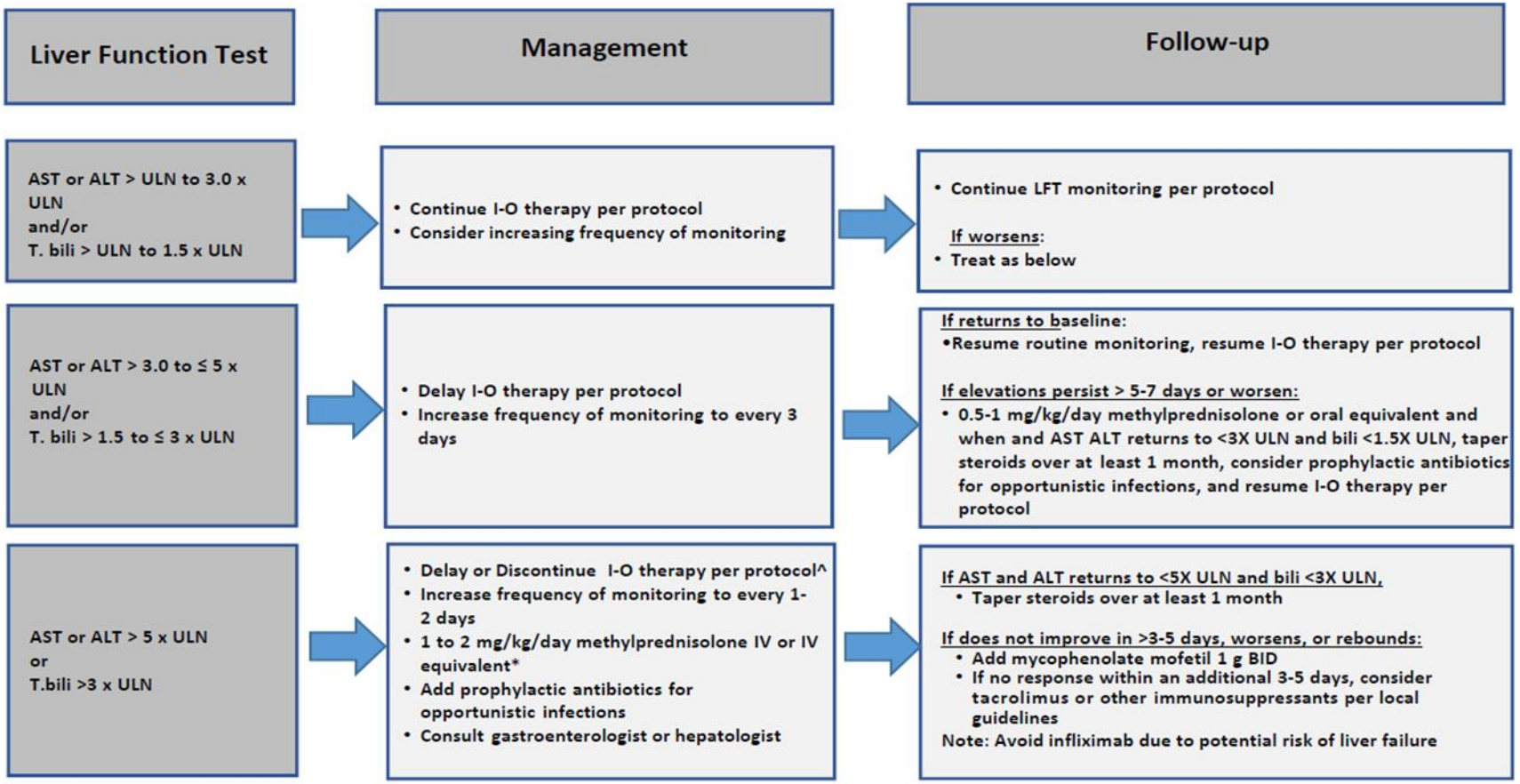
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Evaluate with imaging and pulmonary consultation.




Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

# Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Consider imaging for obstruction.




Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
^ Please refer to protocol dose delay and discontinue criteria for specific details.
*The recommended starting dose for AST or ALT > 20 x ULN or bilirubin >10 x ULN is 2 mg/kg/day methylprednisolone IV.

# Endocrinopathy Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.
Consider visual field testing, endocrinology consultation, and imaging.

| Endocrinopathy | Management | Follow-up |
|---|---|---|
| Asymptomatic TSH abnormality<br>or<br>Hyperglycemia requiring no medical intervention | • Continue I-O therapy per protocol<br>• If TSH < 0.5 x LLN, or TSH > 2 x ULN, or consistently out of range in 2 subsequent measurements:<br>○ include fT4 at subsequent cycles as clinically indicated;<br>○ consider endocrinology consult | • Monitor TSH as per protocol<br>• Monitor blood sugar<br>• If symptomatic or blood sugar requiring initiation of treatment or change in management, manage as below |
| Symptomatic endocrinopathy<br>or<br>hyperglycemia requiring initiation of treatment or change in daily management or work up | • Evaluate endocrine function; consider endocrinology consult<br>• Consider pituitary scan<br>Symptomatic with abnormal lab/pituitary scan:<br>• Delay I-O therapy per protocol<br>• 1-2 mg/kg/day methylprednisolone IV or PO equivalent for hypophysitis<br>• Initiate appropriate hormone/medical therapy<br>No abnormal lab/pituitary MRI scan but symptoms persist:<br>• Continue IO therapy. Repeat labs in 1-3 weeks /MRI in 1 month<br>Hyperglycemia:<br>Screen for Diabetic Ketoacidosis (DKA). If negative, continue IO and treat diabetes. If DKA hold IO, admit, manage DKA. Steroids not recommended for DKA/hyperglycemia | If improves (with or without hormone replacement):<br>• Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections<br>• Resume I-O therapy per protocol<br>• Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component |
| Suspicion of adrenal crisis (eg, severe dehydration, hypotension, shock out of proportion to current illness) | • Delay or discontinue I-O therapy per protocol<br>• Rule out sepsis<br>• Stress dose of IV steroids with mineralocorticoid activity<br>• IV fluids<br>• Consult endocrinologist | • Patients with adrenal insufficiency may need to continue steroids with mineralocorticoid component<br>• If adrenal crisis resolves and patient is adequately controlled with physiologic hormone replacement, patient can resume IO therapy per protocol. |

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

***NOTE, below guidance is for nivolumab given as monotherapy! EO2401*** [redacted]

***DO NOT FOLLOW THE BELOW GUIDANCE FOR ADMINISTRATION SITE REACTIONS RELATED TO EO2040; CONTACT THE MEDICAL MONITOR IN ANY CASE OF DOUBT OF HOW SUCH REACTIONS SHOULD BE HANDLED!***

## Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

| Rash | Management | Follow-up |
|---|---|---|
| Covering ≤ 30% BSA* | • Symptomatic therapy (eg, antihistamines, topical steroids)<br>• Continue I-O therapy per protocol | If persists > 1-2 weeks or recurs:<br>• Consider skin biopsy<br>• Delay I-O therapy per protocol<br>• Consider 0.5-1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving , taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol<br>If worsens:<br>• Treat as >30% BSA |
| Covering > 30% BSA (with or without symptoms)<br><br>Life threatening consequences*^ | • Delay or discontinue I-O therapy per protocol<br>• Consider skin biopsy<br>• Dermatology consult<br>• 1.0 -2.0 mg/kg/day IV methylprednisolone IV or oral equivalent | If improves to < 10% BSA:<br>• Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections<br>• Resume I-O therapy per protocol |

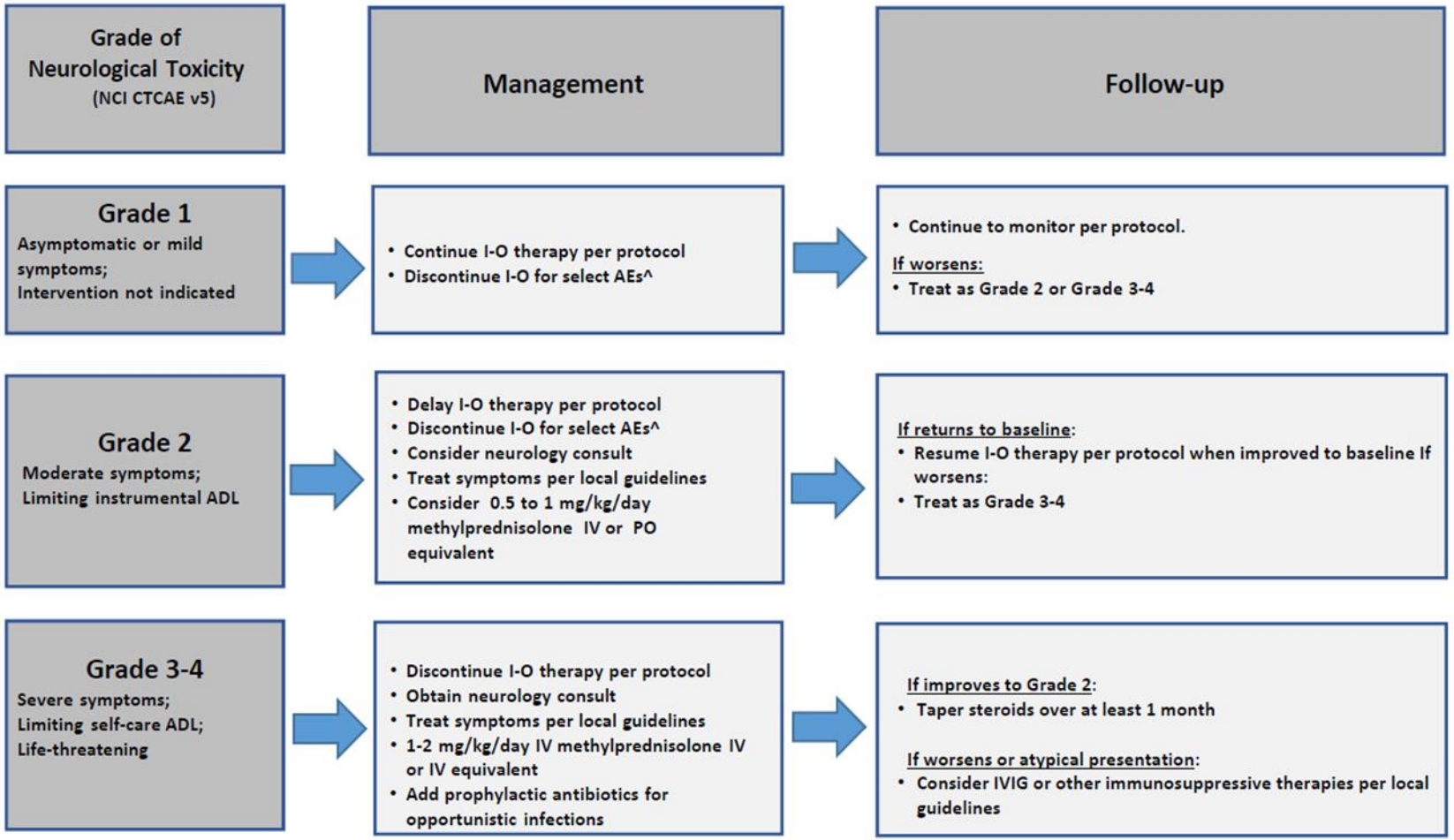
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v5 for term-specific grading criteria.

^If Steven-Johnson Syndrome (SJS),toxic epidermal necrosis (TEN), Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS, TEN, or DRESS is diagnosed, permanently discontinue I-O therapy.

# Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

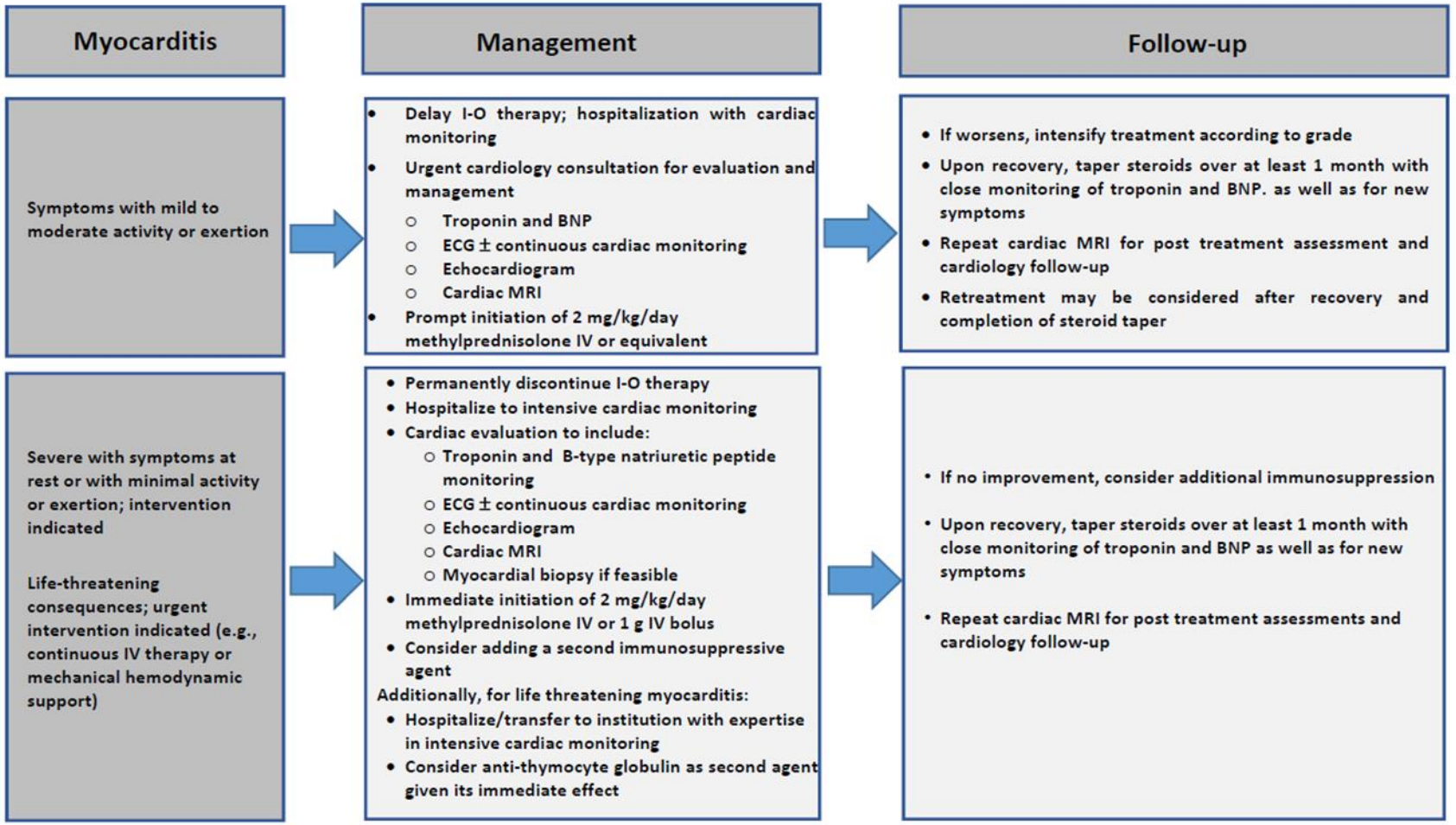



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg. prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

^Discontinue for any grade myasthenia gravis, Guillain-Barre syndrome, treatment-related myelitis, or encephalitis.

# Myocarditis Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.




Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Prophylactic antibiotics should be considered in the setting of ongoing immunosuppression.