



Clinical Trial Protocol EOCRC2-22

A global multicenter phase 1/2 trial of EO4010, a novel microbial-Derived peptide therapeutic vaccine, in combination with nivolumab and/or bevacizumab, for treatment of patients with previously treated metastatic colorectal carcinoma
(the "AUDREY" study)

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Chief Medical Officer

Enterome, Paris, France

Date

Sponsor information

AUDREY Hepburn (4 May 1929 – 20 January 1993) was a British actress and humanitarian. Recognized as both a film and fashion icon, she was ranked by the American Film Institute as the third-greatest female screen legend from the Classical Hollywood cinema and was inducted into the International Best Dressed List Hall of Fame.

Born in Ixelles, Brussels to an aristocratic family, Hepburn spent parts of her childhood in Belgium, England, and the Netherlands. She studied ballet with Sonia Gaskell in Amsterdam beginning in 1945, and with Marie Rambert in London from 1948. She began performing as a chorus girl in West End musical theatre productions and then had minor appearances in several films. She rose to stardom in the romantic comedy *Roman Holiday* (1953) alongside Gregory Peck, for which she was the first actress to win an Oscar, a Golden Globe Award, and a BAFTA Award for a single performance. That year, she also won a Tony Award for Best Lead Actress in a Play for her performance in *Ondine*.

She went on to star in a number of successful films such as *Sabrina* (1954), in which Humphrey Bogart and William Holden compete for her affection; *Funny Face* (1957), a musical where she sang her own parts; the drama *The Nun's Story* (1959); the romantic comedy *Breakfast at Tiffany's* (1961); the thriller-romance *Charade* (1963), opposite Cary Grant; and the musical *My Fair Lady* (1964). In 1967 she starred in the thriller *Wait Until Dark*, receiving Academy Award, Golden Globe, and BAFTA nominations. After that, she only occasionally appeared in films, one being *Robin and Marian* (1976) with Sean Connery. Her last recorded performances were in the 1990 documentary television series *Gardens of the World with Audrey Hepburn* for which she won a Primetime Emmy Award for Outstanding Individual Achievement – Informational Programming.

Later in life, Hepburn devoted much of her time to UNICEF, to which she had contributed since 1954. Between 1988 and 1992, she worked in some of the poorest communities of Africa, South America, and Asia. In December 1992, she received the US Presidential Medal of Freedom in recognition of her work as a UNICEF Goodwill Ambassador. A month later, she died of appendiceal cancer at her home in Switzerland at the age of 63.

**FRANCE* 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.

Dr. Romain Cohen, MD, PhD,

Date

[REDACTED]
[REDACTED]
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Dr. Elena Élez, MD, PhD,

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Dr. Arvind Dasari, MD, MS

Date

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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	Adrenocorticotrophic hormone
AE	Adverse Events
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
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
BSC	Best Supportive Care
BP	Blood Pressure
bpm	Beats per Minute
BSA	Body Surface Area
BSC	Best Supportive Care
BUN	Blood Urea Nitrogen
CAPOX	Capecitabine-oxaliplatin
CDC20	Cell Division Cycle 20
CEA	Carcinoembryonic antigen
CFR	Code of Federal Regulations
CI	Confidence Interval
CK	Creatine Kinase
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
CTLA-4	Cytotoxic T-lymphocyte-associated Antigen 4
DC	Dendritic Cells
DCR	Disease Control Rate
DFS	Disease-free survival
DL	Dose Level
	
DNA	Deoxyribonucleic acid
DOR	Duration Of Response
DP	Drug Product
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
EMA	European Medicines Agency
ENT	Ears, Nose, Throat
ESMO	European Society for Medical Oncology
FACS	Fluorescence-activated Cell Sorting
FAS	Full Analysis Set
FDA	Food and Drug Administration
FIH	First in Human
FOLFOX	Leucovorin/5-fluorouracil/oxaliplatin
FOXM1	forkheadboxM1
FSH	Follicle-stimulating Hormone
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
IL13R α 2	Interleukin-13 Receptor Alpha-2
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
IR	Incidence Rates
IRB	Institutional Review Board
IU	International Unit
IUD	Intra Uterine Device
IUS	Intra Uterine hormone-releasing System
IVS	In Vitro Stimulation
IWRS	Interactive Web Response System
KIF2C	Kinesin Family member 2C
LASR	Local Administration Site Reaction
LDH	Lactate Dehydrogenase
LLN	Lower Limit of Normal
mCRC	Metastatic Colorectal Cancer
MDSCs	Myeloid-derived Suppressor Cells
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major histocompatibility complex
MMR	Mismatch repair
MPP	Malignant Pheochromocytoma/Paraganglioma



mRNA	message Ribonucleic Acid
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
NSCLC	Non-Small-Cell Lung Cancer
NK	Natural Killer
ORR	Overall Response Rate
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PD1	Programmed cell Death protein 1
PDL-1	Programmed Death-Ligand 1
PET	Positron Emission Tomography,
PFS	Progression-free Survival
PI	Principal Investigator
PP	Per-Protocol
PS	Performance Status
PT	Preferred Term
PTT	Partial Thromboplastin Time
RBC	Red Blood Cells
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SARS	Severe Acute Respiratory Syndrome
SC	Subcutaneous
SEM	Standard error of the mean
SmPC	Summary of Product Characteristics
SOC	Standard of Care
SOC	System Organ Class
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
TTR	Time To Response
UBE2C	Ubiquitin conjugating Enzyme E2 C
UCP2	Universal Cancer Peptide 2
ULN	Upper Limit of Normal
USP-NF	United States Pharmacopoeia – National Formulary
VEGF	Vascular Endothelial Growth Factor
WBCs	White Blood Cells
WFI	Water for injections
w/o	Water-in-oil
WT	Wild-type

SYNOPSIS

Full trial title

A global multicenter phase 1/2 trial of EO4010, a novel microbial-derived peptide therapeutic vaccine, in combination with nivolumab and/or bevacizumab, for treatment of patients with previously treated metastatic colorectal carcinoma (*the "AUDREY" study*)

Rationale

Despite all efforts regarding surgery and adjuvant therapy (systemic chemotherapy or targeted treatments), high unmet medical need still exists in patients suffering metastatic colorectal cancer (mCRC). Among people diagnosed with mCRC, approximately 70%-75% of patients survive beyond 1 year, 30%-35% beyond 3 years, and fewer than 20% beyond 5 years from diagnosis. Of note, treatment with the anti-PD1 blocking monoclonal antibodies nivolumab and pembrolizumab are labelled possibilities for patients with mismatch repair (MMR) deficient or high microsatellite instability (MSI-high) metastatic CRC after prior treatment, a group constituting < 5% of patients with metastatic disease. Thus, novel therapeutic approaches are needed to enhance the treatment outcomes for patients with MMR proficient, MSS mCRC. An innovative option proposed with the current trial, is the microbiome-derived peptide therapeutic cancer vaccine approach of EO4010 (composed of five microbial-derived peptides (EO2317, EO2318, OMP10, OMP11, and OMP12) the helper peptide (CD4+ T cell epitope) universal cancer peptide 2 (UCP2), in combination with nivolumab, and/or with bevacizumab in patients with previously treated mCRC.

Objective

- The primary objective of this trial is to evaluate safety and tolerability of EO4010 in combination with nivolumab and/or with bevacizumab in patients with unresectable, previously treated locally advanced or mCRC.
- The secondary objectives include assessment of immunogenicity in relation to T cells of each peptide composing EO4010; T cell cross-reactivity with the human tumor associated antigens (TAAs), objective response rate (ORR), disease control rate (DCR), time to response (TTR), and duration of response (DOR), progression-free survival (PFS) and PFS rate at 4 months, and overall survival (OS).

Main trial endpoints

- The primary endpoint includes safety and tolerability of EO4010 in combination with nivolumab, and/or with bevacizumab 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.
- The secondary trial endpoints include 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 each peptide composing EO4010 by interferon-gamma (IFN- γ) enzyme-linked immunospot (ELISpot), and by intracellular cytokines staining, or multimers staining assays. Cross-reactivities with the human TAAs. ORR, DCR, TTR, DOR and PFS as described by RECIST 1.1 and iRECIST criteria. OS defined as the time interval from the date of first study treatment administration to the date of death due to any cause.

Trial design

This is an open-label, multicenter, FIH, phase 1/2 trial to assess safety, tolerability, immunogenicity, and preliminary efficacy of the microbial-derived therapeutic vaccine EO4010 in combination with nivolumab and/or with bevacizumab, for treatment of patients with unresectable, previously treated, metastatic CRC.

The trial is a 3-cohort study intended to recruit a maximum of 42 evaluable patients in total:

- Cohort 1 includes a safety evaluation by a [REDACTED] of EO4010 monotherapy ([REDACTED]) planned to be followed in the same individual patients by continued EO4010 in combination with nivolumab (latter at standard doses per registrations in other settings); 3 to 12 evaluable patients will be included depending on the safety profile of the administered treatments.
- Cohort 2 includes an evaluation of EO4010 at the recommended dose found in Cohort 1 in combination with nivolumab in 15 evaluable patients with CRC.
- Cohort 3 (3a and 3b) will be run once Cohort 2 recruitment is completed. Cohort 3a includes an evaluation of EO4010 in combination with nivolumab and bevacizumab in 10 evaluable patients with

CRC, Cohort 3b includes an evaluation of EO4010 in combination with bevacizumab in 5 evaluable patients with CRC.

The assumed recruitment time is [REDACTED]. The draft 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 assumed to be 12 months after the last patient enrolled; this time is an estimate and might change (no protocol amendment is assumed if within the expected margins of 12 to 24 months after the last patient enrolled; see [section 4.3.1](#)). The trial duration from start of recruitment until last patient visit is assumed to be maximum 42 months.

Trial population

The study is assumed to recruit approximately maximum of 42 evaluable patients with the following main inclusion/exclusion criteria (not exhaustive; please refer to trial protocol for the full list):

- Main inclusion criteria:
 - HLA-A2 positive patients with advanced non-resectable colorectal adenocarcinoma which is mismatch repair proficient and microsatellite stable, who have been previously treated with, or are not considered candidates therapies including fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapies, anti-VEGF agents, and anti-EGFR agents; Progression during or within 3 months following the latest administration of standard therapies (outlined above) at an age ≥ 18 years with ECOG performance status 0 to 1.
- Main exclusion criteria:
 - patients treated with dexamethasone > 2 mg/day or equivalent (i.e., 13 mg/day of prednisone) within 14 days before first administration of EO4010; who have received any prior treatment with compounds targeting PD1, PD-L1, CTLA-4, or similar compounds, trifluridine/tipiracil (TAS-102) or regorafenib; 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; with persistent Grade ≥ 2 toxicities (according to NCI-CTCAE v5.0), except alopecia, neuropathy, and other persisting toxicities not constituting a safety risk based on Investigator's judgment; with uncontrolled central nervous system (CNS) metastasis, with the significant abnormal laboratory values hematology, liver and renal function, 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. Patients with a history of solid organ transplantation or allogeneic hematopoietic stem cell transplantation, history or presence of human immunodeficiency virus (HIV) and/or active hepatitis B virus (HBV)/hepatitis C virus (HCV).

Interventions

- Patients included in Cohort 1 (a safety evaluation by a 3-by-3 design of EO4010 monotherapy) will receive subcutaneous administration of EO4010 (i.e. 1.8 mg/1 mL of EO4010 [REDACTED] monotherapy starting from week 1 (W1) (2 administrations of EO4010 each followed by [REDACTED] observation period, followed by EO4010 combination with nivolumab 240 mg [REDACTED] and onwards ([REDACTED] EO4010+ nivolumab 480 mg [REDACTED]).
- Patients included in Cohort 2 will receive EO4010 (given SC together [REDACTED] in combination with nivolumab 240 mg (every 2 weeks from W1 to W5), onwards (from W7) EO4010 + nivolumab 480 mg [REDACTED]).
- In Cohort 3 (3a and 3b) 10 patients will be treated by EO4010 with nivolumab and bevacizumab (3a arm) and 5 patients will be treated by EO4010 with bevacizumab (3b arm). EO4010 and nivolumab will be administered at the same doses and at the same time points that in Cohort 2, bevacizumab will be administered at [REDACTED] from [REDACTED].

Study treatments will be administered until confirmed tumor progression (iRECIST criteria for confirmed tumor progression are to be used), intolerable toxicity, death, reached maximum individual patient study treatment duration [REDACTED], patient or Investigator decision, or planned or early termination of the study. Specific regular safety monitoring of blood values, physical examination, and ECG are implemented as well as monitoring of all adverse events. The disease is followed by CT scan. Stool and blood samples are collected for research purposes.

**Ethical considerations relating to the clinical trial including the expected benefit to the individual subject or group of patients represented by the trial subjects as well as the nature and extent of burden and risks**

This trial aims at assessing the safety and the preliminary efficacy of EO4010 in combination with nivolumab and/or with bevacizumab in patients with non-resectable locally advanced or metastatic CRC previously treated with standard chemotherapy +/- targeted therapy. EO4010 induces an efficacious expansion of T cells specific for the targeted TAAs with ability to kill tumor cells expressing these TAAs and may potentially lead to an anti-tumor response. EO4010 has demonstrated good tolerability in animal models and a good safety profile in 3 patients treated in the safety lead-in part of this trial (Cohort 1). Dedicated monitoring is applied for adverse events that might occur, based on USPI/EU SmPCs for the nivolumab and bevacizumab and based on the selection of antigens targeted by EO4010 and its method of administration. The main anticipated adverse events caused by EO4010 are mild to moderate local administration site reactions (redness, local pain, swelling, inflammation). The selected peptides included in EO4010 have been shown to induce strong immune responses in non-clinical models and in the first 3 treated patients. Hence, the benefit/risk ratio for the proposed therapeutic-vaccination approach is considered to be positive.



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 (Argilés et al., 2020).

In 2020, an estimated 104,610 new cases of colon cancer and 43,340 cases of rectal cancer will occur in the USA (*NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 1, 2022, 2022*). 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 (*NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 1, 2022, 2022*).

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 (Argilés et al., 2020).

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. Individuals 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 (*NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 1, 2022, 2022*).



1.1.2 Curative treatment settings

After appropriate diagnostic measures surgical intervention is the first key step in treatment of colon cancer (Argilés et al., 2020; *NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 1, 2022, 2022*).

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 (Argilés et al., 2020).

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 (Argilés et al., 2020). In addition, for intermediate stage II, further parameters need consideration to refine the evaluation of risk given the observed variability on prognosis (Argilés et al., 2020).

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 (Argilés et al., 2020).

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 (Argilés et al., 2020). 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 (Argilés et al., 2020; Baxter et al., 2022; *NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 1, 2022, 2022*).

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 (*NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 1, 2022, 2022*). 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% (*NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 1, 2022, 2022*). 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 (*NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 1, 2022, 2022*).



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 ([NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 4, 2023](#)):

- 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.

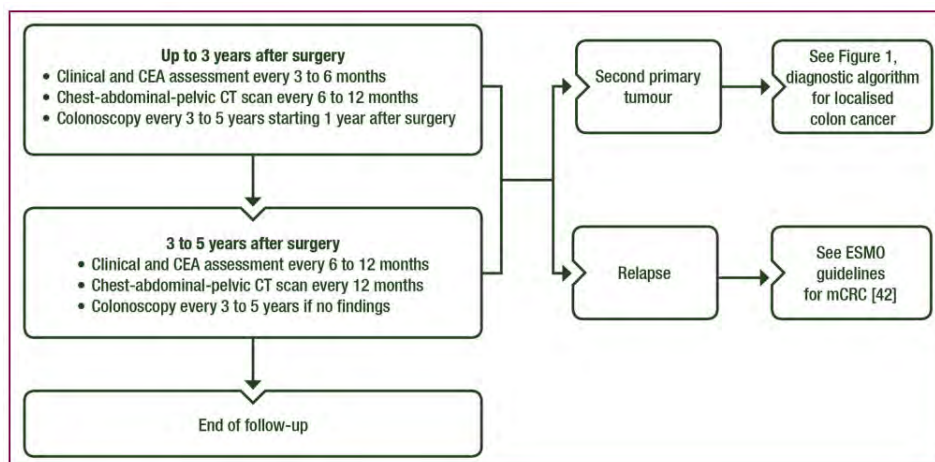
The NCCN panel recommendation for surveillance of patients with stage IV CRC with no evidence of disease (NED) after curative-intent surgery and subsequent adjuvant treatment are similar to those listed for patients with stage II/III disease, except that certain evaluation are performed more frequently. 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. 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 ([NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 4, 2023](#)).

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 ([NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 4, 2023](#)).

The ESMO guideline regarding localized colon cancer is in principle similar to the NCCN panel recommendations for stage II/III disease (**Figure 1** ([Argilés et al., 2020](#))). 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 ([Argilés et al., 2020](#); [Biller & Schrag, 2021](#)).

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 (Biller & Schrag, 2021). In addition, of new colorectal cancer diagnoses, 20% of patients have metastatic disease already at presentation (Biller & Schrag, 2021).

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. 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 (NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 4, 2023).

Metastatic disease not amenable to local treatment only (e.g. surgery, stereotactic body radiation therapy, hepatic 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 (Argilés et al., 2020; NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 4, 2023).

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 (NCCN Clinical Practice Guidelines in Oncology, Colon Cancer, Version 4, 2023). 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.

Of note, treatment with the anti-PD1 blocking monoclonal antibodies nivolumab and pembrolizumab are labelled possibilities for patients with MMR deficient or MSI-high



metastatic CRC after prior treatment, a group constituting < 5% of patients with metastatic disease (Biller & Schrag, 2021).

As outlined in the ESMO guidance regarding metastatic CRC (Van Cutsem et al., 2016), 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 (Biller & Schrag, 2021).

The following European Society for Medical Oncology (ESMO) 2023 Living Guideline has been recently updated with new treatment recommendations: use of TAS-102+bevacizumab as third-line treatment for metastatic colorectal cancer (Cervantes et al., 2023)

Thus, as outlined above, despite of new treatment combination availability, CRC continues to be a major therapeutic challenge with a considerable number of patients experiencing premature death due to disease recurrence indicating that novel treatment concepts are needed.

1.1.5 Regorafenib and trifluridine/tipiracil (TAS-102)

Considering the target population of the current study it is of interest to consider the outcome of the label enabling trials for regorafenib (Stivarga) and trifluridine/tipiracil (TAS-102; Lonsurf) which are described below and considered relevant benchmarks for the current trial (Grothey et al., 2013; Mayer et al., 2015).

Regorafenib (Stivarga) is a small molecule inhibitor of multiple membrane-bound and intracellular kinases involved in normal cellular functions and in pathologic processes registered for the treatment of patients with metastatic CRC who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF therapy, and, if KRAS wild type, an anti-EGFR therapy.

The clinical efficacy and safety of regorafenib were evaluated in an international, multi-center, randomized (2:1), double-blind, placebo-controlled trial in 760 patients with previously treated metastatic CRC (Grothey et al., 2013). Patients were randomized to receive regorafenib orally once daily (n=505) plus Best Supportive Care (BSC) or placebo (n=255) plus BSC for the first 21 days of each 28-day cycle. Treatment continued until disease progression or unacceptable toxicity. In the all-randomized population, median age was 61 years, 61% were men, 78% were White, and all patients had baseline ECOG performance status of 0 or 1. The primary site of disease was colon (65%), rectum (29%), or both (6%). History of KRAS evaluation was reported for 729 (96%) patients; 430 (59%) of



these patients were reported to have KRAS mutation. The median number of prior lines of therapy for metastatic disease was 3. All patients received prior treatment with fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy, and with bevacizumab. All but one patient with KRAS mutation-negative tumors received panitumumab or cetuximab. Outcome measures of the trial are outlined in the table and figures (OS followed by PFS) below.

Table 1: Overall survival, progression-free survival, and overall response rate for the phase 3 regorafenib (Stivarga) trial ([Stivarga-Drugs@FDA, n.d.](#))

	Stivarga + BSC (N=505)	Placebo + BSC (N=255)
Overall Survival		
Number of deaths, n (%)	275 (55%)	157 (62%)
Median Overall Survival (months)	6.4	5.0
95% CI	(5.8, 7.3)	(4.4, 5.8)
HR (95% CI)	0.77 (0.64, 0.94)	
Stratified Log-Rank Test P-value ^{a,b}	0.0102	
Progression-free Survival		
Number of Death or Progression, n (%)	417 (83%)	231 (91%)
Median Progression-free Survival (months)	2.0	1.7
95% CI	(1.9, 2.3)	(1.7, 1.8)
HR (95% CI)	0.49 (0.42, 0.58)	
Stratified Log-Rank Test P-value ^a	<0.0001	
Overall Response Rate		
Overall response, n (%)	5 (1%)	1 (0.4%)
95% CI	0.3%, 2.3%	0%, 2.2%

^a Stratified by geographic region and time from diagnosis of metastatic disease.
^b Crossed the O'Brien-Fleming boundary (two-sided p-value < 0.018) at second interim analysis.

Figure 2: Overall survival for regorafenib vs placebo by Kaplan-Meier analysis of intention-to-treat population ([Grothey et al., 2013](#))

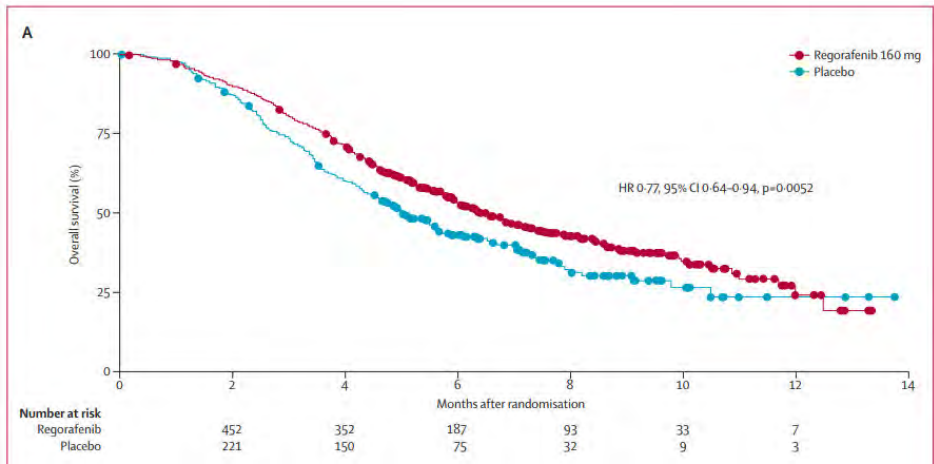
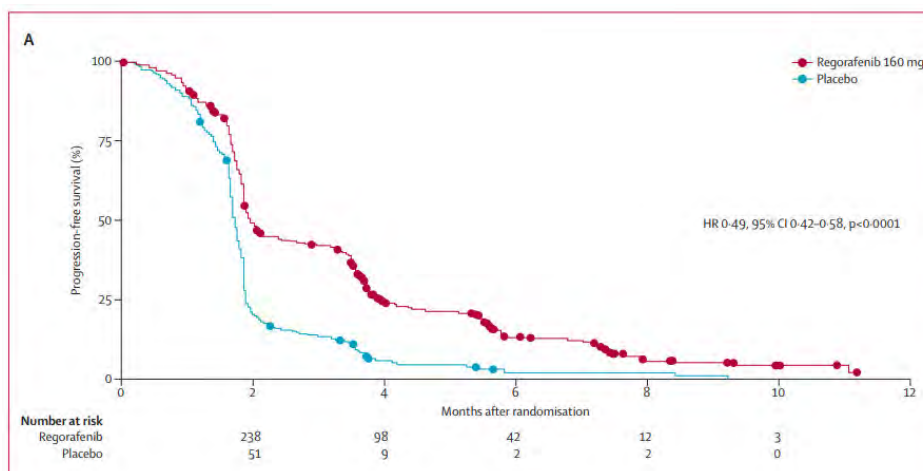




Figure 3: Progression-free survival for regorafenib vs placebo by Kaplan-Meier analysis of intention-to-treat population (Grothey et al., 2013)



Trifluridine and tipiracil (TAS-102; Lonsurf) is a combination of a nucleoside metabolic inhibitor (trifluridine) and a thymidine phosphorylase inhibitor (tipiracil), registered for the treatment of adult patients with metastatic colorectal cancer who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF biological therapy, and if RAS wild-type, an anti-EGFR therapy.

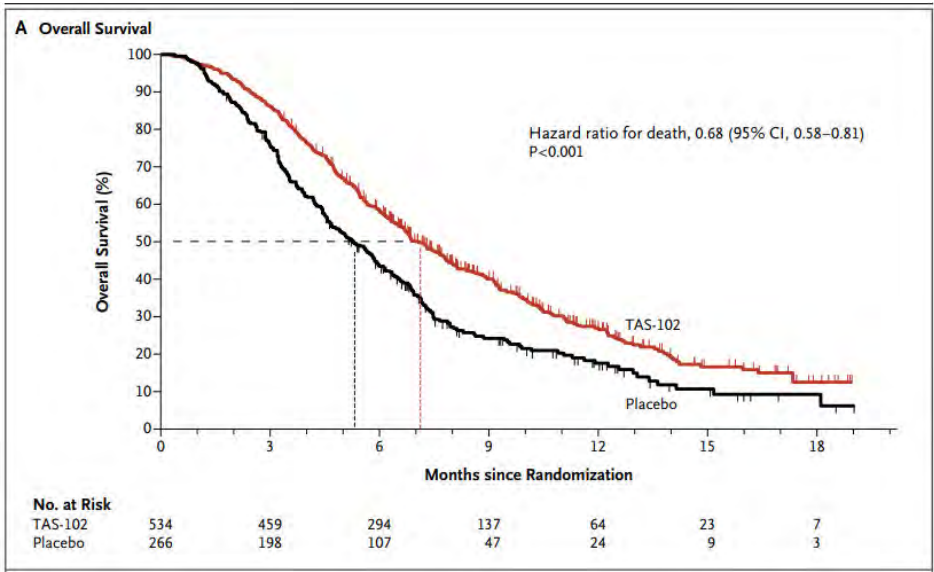
The efficacy of TAS-102 was evaluated in RECURSE (NCT01607957), an international, randomized, double-blind, placebo-controlled study conducted in patients with previously treated metastatic CRC (Mayer et al., 2015). Key eligibility criteria included prior treatment with at least 2 lines of standard chemotherapy for metastatic CRC, ECOG performance status (PS) 0-1, absence of brain metastasis, and absence of ascites requiring drainage in the past four weeks. Patients were randomized 2:1 to receive TAS-102 or matching placebo orally twice daily on days 1-5 and 8-12 of each 28-day cycle until disease progression or unacceptable toxicity. Randomization was stratified by KRAS status (wild type vs. mutant), time since diagnosis of first metastasis (< 18 months vs $\geq$ 18 months), and region (Japan vs US, Europe and Australia).

A total of 800 patients were randomized to TAS-102 (N=534) with best supportive care (BSC) or matching placebo (N=266) plus BSC. The median age was 63 years, 61% were male, 58% and 35% were White and Asian, respectively, and all patients had baseline ECOG PS of 0 or 1. The primary site of disease was colon (62%) or rectum (38%). KRAS status was wild-type (49%) or mutant (51%) at study entry. All patients received prior treatment with fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy. All but one patient received bevacizumab and all but two patients with KRAS wild-type tumors received panitumumab or cetuximab.

At the time that the target number of events was reached (574 deaths), the median overall survival was 7.1 months (95% confidence interval [CI], 6.5 to 7.8) in the TAS-102 group and 5.3 months (95% CI, 4.6 to 6.0) in the placebo group. The hazard ratio for death (TAS-102 vs placebo) was 0.68 (95% CI, 0.58 to 0.81; $P<0.001$). The 1-year survival rates were 27% and 18%, respectively. Three factors were identified as prognostic: time since diagnosis of first metastasis, ECOG performance status, and number of metastatic sites. However, the magnitude of the TAS-102 treatment effect, after adjustment for all three factors, was maintained (hazard ratio, 0.69; 95% CI, 0.58 to 0.81).



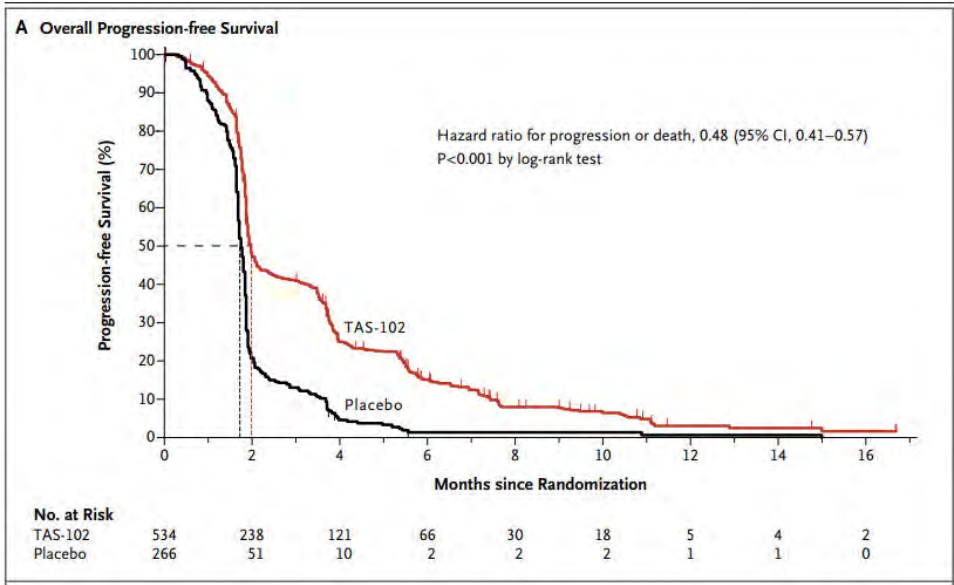
Figure 4: Overall survival for TAS-102 vs placebo by Kaplan-Meier analysis (Mayer et al., 2015)



The median progression-free survival was 2.0 months (95% CI, 1.9 to 2.1) in the TAS-102 group and 1.7 months (95% CI, 1.7 to 1.8) in the placebo group. The hazard ratio for progression (TAS-102 vs placebo) was 0.48 (95% CI, 0.41 to 0.57; P<0.001).

In the tumor-response population (502 patients in the TAS-102 group and 258 in the placebo group), 8 patients in the TAS-102 group had a partial response, and 1 patient in the placebo group was reported to have a complete response, resulting in objective response rates of 1.6% with TAS-102 and 0.4% with placebo (P=0.29). Disease control (complete or partial response or stable disease, assessed at least 6 weeks after randomization) was achieved in 221 patients (44%) in the TAS-102 group and 42 patients (16%) in the placebo group (P<0.001).

Figure 5: Progression-free survival for TAS-102 vs placebo by Kaplan-Meier analysis (Mayer et al., 2015)





1.1.6 Trifluridine/tipiracil (TAS-102 or FTD-TPI) in combination with bevacizumab

The efficacy of TAS-102 in combination with bevacizumab was evaluated in SUNLIGHT (NCT04737187), an international, open-label, controlled two-arm, randomized phase III comparison study (TAS-102 + Bevacizumab vs TAS-102) in patients with refractory mCRC (Prager et al., 2023). Key eligibility criteria included patients with prior treatment with no more than two previous chemotherapy regimens for the treatment of advanced CRC and who had had progressive disease or if their last regimen had caused unacceptable adverse effects. Patients had to be 18 years of age or older and have adequate organ function and ECOG performance status (PS) 0-1, Eligibility required knowledge of RAS status.

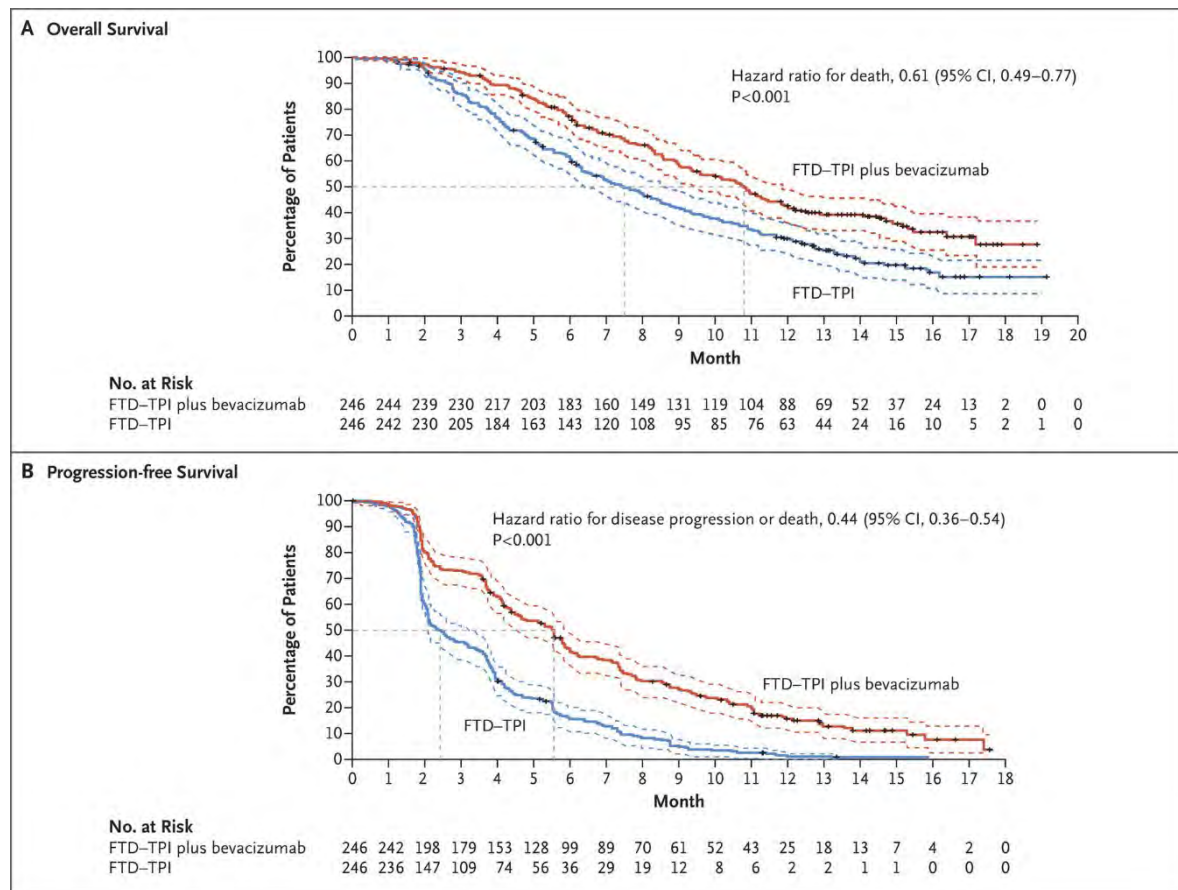
Overall, 492 patients underwent randomization. A total of 246 patients were assigned to each group. The percentage of patients with RAS mutations (69.3%) was higher than that in the general population of patients with metastatic colorectal cancer, potentially reflecting preferential referral of patients with RAS wild-type tumors to clinical trials of antiEGFR therapy. Most patients (92.1%) had received two previous treatment regimens for metastatic disease; however, 4.5% of the patients in the combination group and 6.1% in the FTD-TPI group had received only one first-line triplet regimen, and 2.6% of the patients in the trial had received three or more previous drug regimens for metastatic disease.

The median follow-up was 14.2 months (interquartile range, 12.6 to 16.4) in the combination group and 13.6 months (interquartile range, 12.7 to 15.9) in the TAS-102 group. The median overall survival was 10.8 months (95% confidence interval [CI], 9.4 to 11.8) in the combination group and 7.5 months (95% CI, 6.3 to 8.6) in the TAS-102 group (hazard ratio for death, 0.61; 95% CI, 0.49 to 0.77; $P < 0.001$) (Fig. 6). A sensitivity analysis that excluded patients who did not meet relevant prespecified medical and therapeutic criteria (14 patients in the combination group and 16 patients in the TAS-102 group) showed a median overall survival of 10.8 months (95% CI, 9.6 to 12.1) in the combination group and 7.2 months (95% CI, 6.3 to 8.5) in the TAS-102 group (hazard ratio for death, 0.59; 95% CI, 0.47 to 0.74). The 6-month overall survival was 77% in the combination group and 61% in the TAS-102 group; the 12-month overall survival was 43% and 30%, respectively (Figure 6). The median progression free survival was 5.6 months (95% CI, 4.5 to 5.9) in the combination group and 2.4 months (95% CI, 2.1 to 3.2) in the TAS-102 group (hazard ratio for disease progression or death, 0.44; 95% CI, 0.36 to 0.54; $P < 0.001$). The 6-month progression-free survival was 43% in the combination group and 16% in the TAS-102 group; the 12-month progression-free survival was 16% and 1%, respectively (Figure 6). An objective response was observed in 6.1% of the patients in the combination group (95% CI, 3.5 to 9.9), with 15 patients having had a partial response, and in 1.2% of the patients in TAS-102 group (95% CI, 0.3 to 3.5), with 1 patient having had a complete response and 2 patients having had a partial response (between group difference, 4.9 percentage points; 95% CI, 1.6 to 8.2).

Adverse events (AEs) of any cause occurred in 98.0% of the patients in each group. Severe AEs (grade ≥ 3) were reported in 72.4% of the patients in the combination group and in 69.5% of the patients in the TAS-102 group. Serious AEs were reported in 24.8% and 31.3% in the two groups, respectively. The most common AEs that occurred during the treatment period in both groups were neutropenia, nausea, and anemia. The events that were more frequent in the combination group than in the TAS-102 group were hypertension (in 10.2% of the patients in the combination group and 2.0% in the FTD-TPI group), nausea (in 37.0% and 27.2%, respectively), and neutropenia (in 62.2% and 51.2%, respectively), including severe (grade ≥ 3) neutropenia (in 43.1% and 32.1%, respectively).

Median time to deterioration in global health status was 8.5 months vs 4.7 months (HR=0.50; $P<.001$) and worsening to ECOG PS ≥ 2 was delayed, with median time of 9.3 months vs 6.3 months respectively (HR=0.54; $P<.001$).

Figure 6: Overall Survival and Progression-free Survival (Intention-to-Treat Population) (Prager et al., 2023)



Treatment with TAS-102 plus bevacizumab resulted in significantly longer overall survival and progression-free survival and better disease control than treatment with TAS-102 alone. The addition of bevacizumab to TAS-102 did not increase the risk of serious adverse events or adverse events leading to treatment discontinuation. Measurement of quality of life also showed the advantage of adding bevacizumab. Clinical benefit was also observed regardless of whether patients had received previous treatment with bevacizumab, a finding that adds to the body of evidence supporting a role for continued inhibition of angiogenesis beyond progression and suggesting that the addition of bevacizumab to third-line or later-line therapy may prolong survival in heavily pretreated patients.

Considering the target population of the current study it is of interest as well to consider the outcomes of SUNLIGHT study which are described above and considered as a new relevant benchmark for the current trial.

1.1.7 Fruquintinib

The FRESCO-2 study which is described below might also be considered as a benchmark (Dasari et al., 2023).



FRESCO-2 study (NCT04322539) is the international, randomised, double-blind, placebo-controlled, phase III evaluating the efficacy and safety of fruquintinib among patients aged 18 years or older (≥ 20 years in Japan) with histologically or cytologically documented metastatic colorectal adenocarcinoma who had received all current standard approved cytotoxic and targeted therapies and progressed on or were intolerant to trifluridine-tipiracil or regorafenib, or both.

Eligible patients were randomly assigned 2:1 to receive fruquintinib 5 mg capsule or matched placebo orally once daily on days 1-21 in 28-day cycles, plus best supportive care. Stratification factors were previous trifluridine-tipiracil or regorafenib, or both, *RAS* mutation status, and duration of metastatic disease. The primary endpoint was OS, defined as the time from randomisation to death from any cause. A non-binding futility analysis was done when approximately one-third of the expected OS events had occurred. Final analysis occurred after 480 OS events.

Overall, 691 patients underwent randomization of whom 461 were randomly assigned to receive fruquintinib and 230 to placebo. Patients had received a median of 4 lines of previous systemic treatment for metastatic disease, and 502 of 691 patients (73%) had received more than 3 lines.

Median OS was 7.4 months in the fruquintinib group versus 4.8 months in the placebo group (hazard ratio [HR] 0.66, 95% CI 0.55–0.80; $p < 0.0001$). The Kaplan-Meier plot for OS showed an early separation of the curves in favour of the fruquintinib group, which was maintained over the duration of the study. Median PFS was 3.7 months in the fruquintinib group versus 1.8 months in the placebo group (HR 0.32, 95% CI 0.27–0.39; $p < 0.0001$).

Subgroup analyses of OS and PFS showed results that were consistent with the benefit observed in the intention-to-treat population across nearly all prespecified subgroups, including by randomisation stratification factors of previous treatment with trifluridine-tipiracil or regorafenib, *RAS* mutation status, and duration of metastatic disease. Furthermore, OS and PFS benefits were also consistent regardless of number of previous lines of treatment for metastatic disease.

Investigator-assessed objective response was documented in 7 of 461 patients (2%) in the fruquintinib group compared with none in the placebo group (adjusted difference 2%, 95% CI 0.4–2.7; $p = 0.059$). No complete responses were observed. The median duration of response (DoR) in the fruquintinib group was 10.7 months and maximum DoR was ongoing at greater than 16.9 months. In the fruquintinib group, DCR was 56% compared with 16% in the placebo group (adjusted difference 39%, 95% CI 32.8–46.0; $p < 0.0001$).

Grade 3 or worse adverse events occurred in 286 of 456 patients (63%) who received fruquintinib and 116 of 230 patients (50%) who received placebo. The most common grade 3 or worse adverse events in the fruquintinib group included hypertension (14%), asthenia (8%), and hand-foot syndrome (6%). There was one treatment-related death in each group, intestinal perforation in the fruquintinib group and cardiac arrest in the placebo group.

FRESCO-2 is the first study to show efficacy of an oral VEGFR inhibitor in patients who have received previous trifluridine-tipiracil or regorafenib, or both. The observed reduction in the overall risk of death and risk of progression or death with fruquintinib compared with placebo suggests that inhibition of the VEGF pathway remains an effective management strategy for mCRC, even in the later line setting and in patients with previous exposure to antiangiogenic agents. The findings from FRESCO-2 show that fruquintinib is effective and well tolerated in a broad population of patients with heavily pretreated mCRC.



The FRESCO-2 study support the clinical relevance of angiogenesis inhibition even in the more advanced lines of treatment.

1.2 EO4010 a microbiome-derived therapeutic vaccine for treatment of colorectal cancer

Summarized information about the EO4010 compound, and its targets are presented below; for extensive description see the current Investigator's Brochure (IB) of EO4010 further describing the characteristics of EO4010.

Extensive pre-clinical studies have been conducted to characterize the microbiome-derived mimicry therapeutic vaccine concept (**Figure 7**), 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.

EO4010, the compound to be used in the current trial includes two components from the compound EO2401, *i.e.* the peptides EO2317 and EO2318, mimicking the TAAs BIRC5 and FOXM1.

Of note, the compound EO2401 in combination with nivolumab +/- bevacizumab has been investigated in two phase 1/2 trials in 170 patients with glioblastoma and adrenal tumors, showing the ability to generate strong systemic immune responses against the targeted TAAs correlating with clinical outcome (progression-free survival and objective response), and being well tolerated; the safety profile being consistent with the profile of the combination partners nivolumab and bevacizumab, 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.

EO4010, 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:

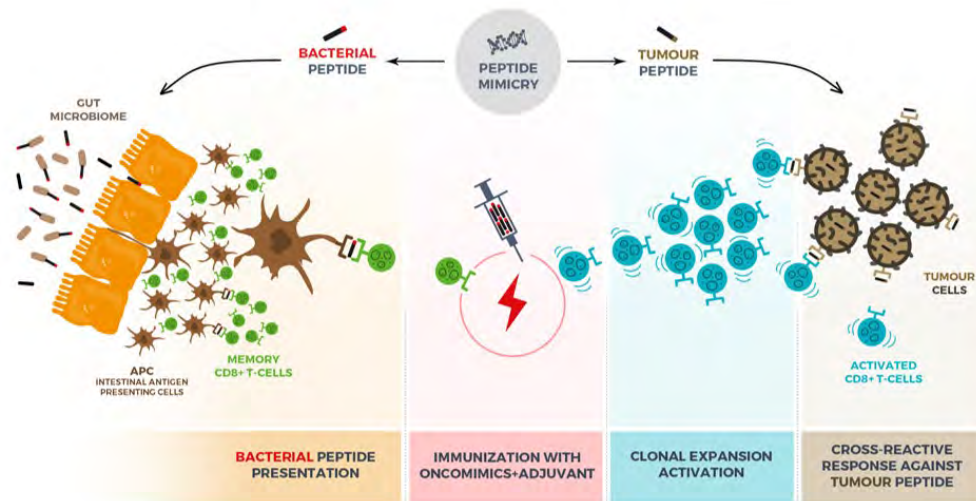
- high affinity MHC class I binding-peptides (*i.e.* targeting efficacious presentation for cytotoxic CD8⁺ T cells),
- high capacity to be recognized by the immune system by its non-self-nature, and

- 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 EO4010 and an anti-PD1 blocking agent is utilized to optimize the fast expansion of specific T cells without negative feed-back loops, and possibility for tumor specific T cells to infiltrate and effectively function within the tumor environment. The concomitant administration of EO4010 and VEGF/VEGFR blocking agent is utilized to increase tumor infiltration of T cells via vasculature normalization, and to activate the effector immune cells through stimulation of the maturation of dendritic cells (DCs) and reduction of the expansion of Tregs and myeloid-derived suppressor cells (MDSCs) (see further [Section 1.2.5](#)).

Figure 7: Microbiome-derived peptide therapeutic vaccine concept



EO4010 Drug Product (DP; not adjuvanted) is a solution of five synthetic 9-amino acid microbiome-derived peptides (EO2317, EO2318, OMP10, OMP11, and OMP12), and the synthetic 15-amino acid helper peptide (Universal Cancer Peptide 2, UCP2) in a water for injections (WFI) [REDACTED]. The peptide mixture solution EO4010 (final concentration of each peptide is 300 µg/mL in emulsion), will extemporaneously be [REDACTED] d with the [REDACTED] to form a water-in-oil emulsion before subcutaneous (SC) administration. EO4010 will also be given in combination with the anti-PD1 monoclonal antibody nivolumab and/or with bevacizumab, administered according to global labels.

The microbiome-derived therapeutic vaccine concept utilized in conjunction with anti-PD1 blockade is an innovative option for testing of a rationally developed immunotherapy for CRC. The concept as such, including the combination with nivolumab, has already been tested in the clinical setting 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.

Thus, the current study will evaluate the microbiome-derived therapeutic vaccine EO4010 in combination with nivolumab and/or in combination with bevacizumab in patients with previously treated metastatic colorectal carcinoma.

1.2.1 The microbiome derived peptides in EO4010

Summarized information about the five microbiome derived peptides included in the EO4010 compound are presented below; for an extensive description and justification of the use of each of the individual peptides (including normal tissue expression [lack of], overexpression in colorectal tumors, and correlations with clinicopathological features of colorectal tumors) see the current IB of EO4010.

The EO2317, EO2318, OMP10, OMP11, and OMP12 peptides included in EO4010 were found in the human microbiota, are restricted to HLA-A2 expressed in 49% of the Caucasian population (Chen et al., 2012) and display high sequence homologies with five different TAAs: survivin also called baculoviral inhibitor of apoptosis repeat-containing protein 5 (BIRC5), forkhead box M1 (FOXM1), ubiquitin conjugating enzyme E2 C (UBE2C; also called UBCH10), cell division cycle 20 (CDC20), and kinesin family member 2C (KIF2C; also called MCAK).

These TAAs are pan-tumor associated proteins, all with critical cell-cycle functions (Figure 8), and highly expressed in CRC but not relevantly in normal tissues (Figure 9 & Figure 10).

The peptides demonstrated in pre-clinical testing high MHC binding affinity, strong immune responses as well as cross reactivity against the human corresponding peptides (see the current IB of EO4010).

The five different TAAs were selected to overcome possible tumor heterogeneity and reduce tumor escape.

Figure 8: Implication of BIRC5, KIF2C, FOXM1, UBE2C and CDC20 in different cell cycle processes [adapted from (Schibler et al., 2009)]

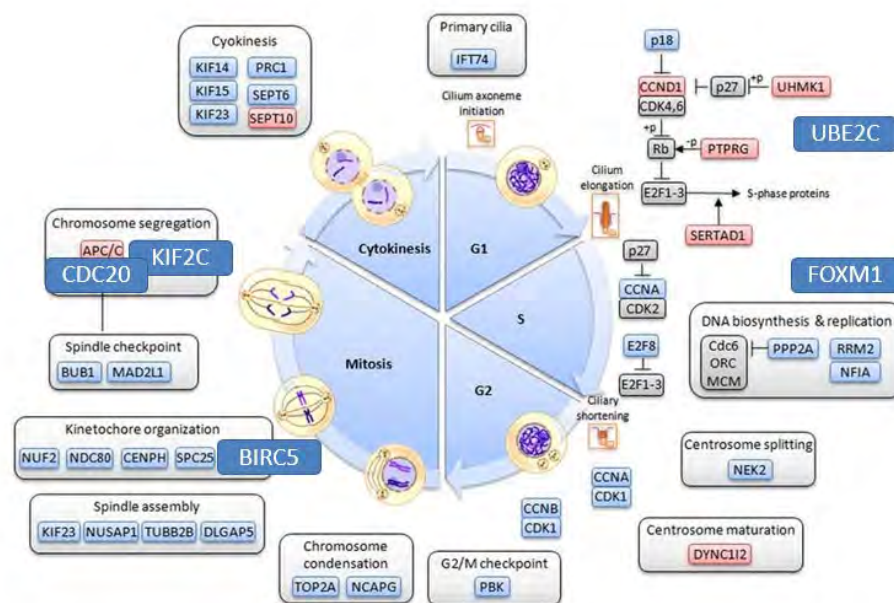




Figure 9: Median relative mRNA expression of BIRC5, KIF2C, FOXM1, UBE2C and CDC20 mRNA in tumor and normal samples (RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects, GEPIA database)

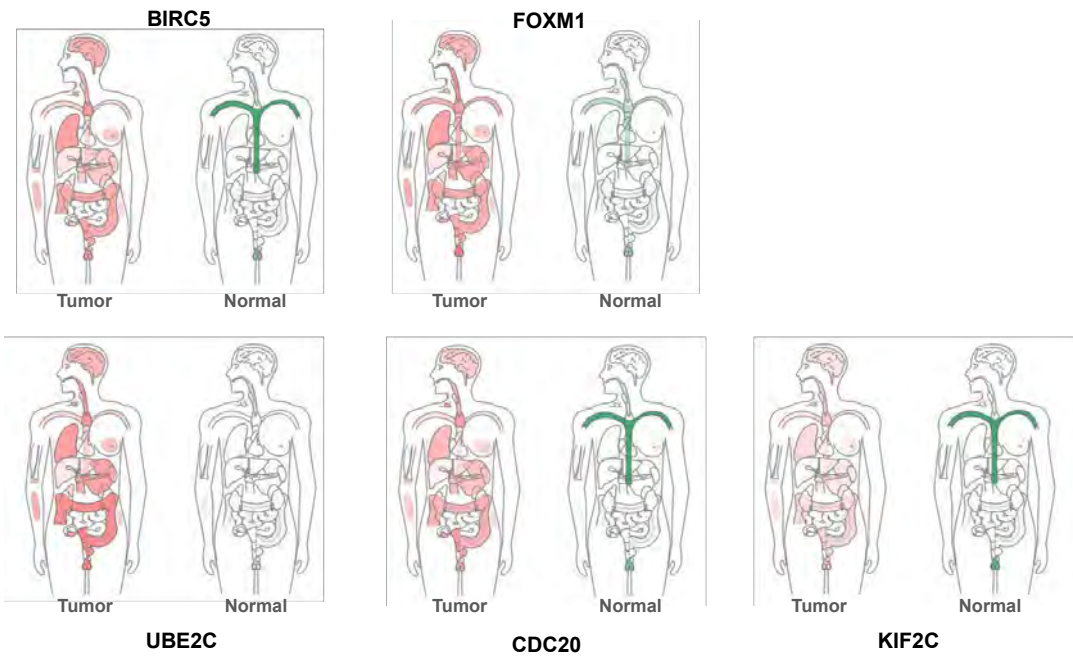
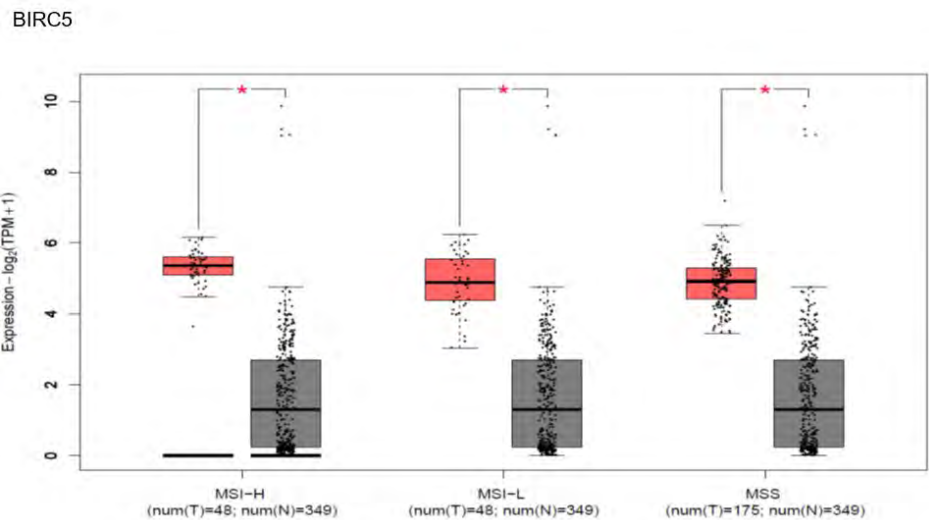
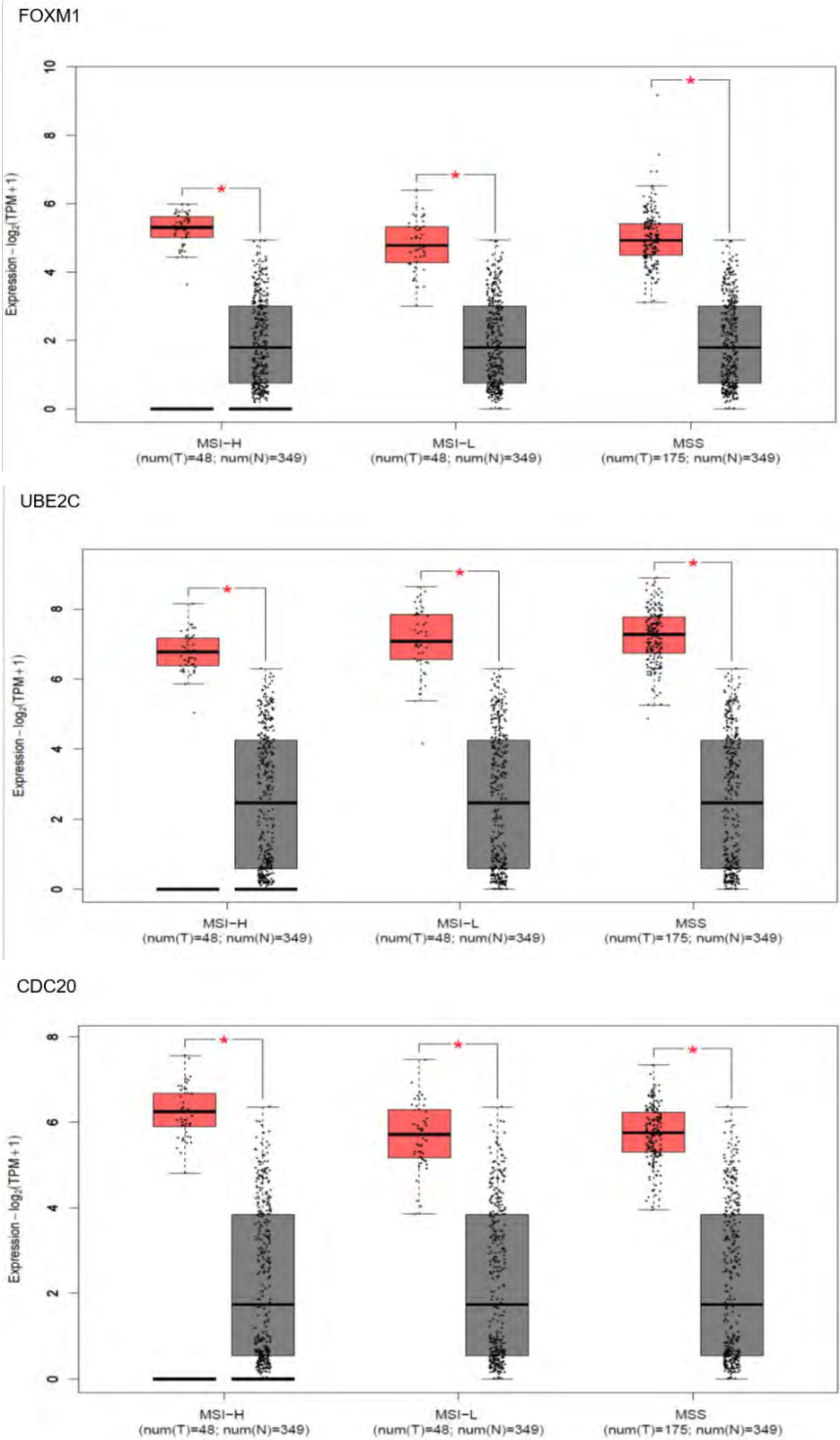
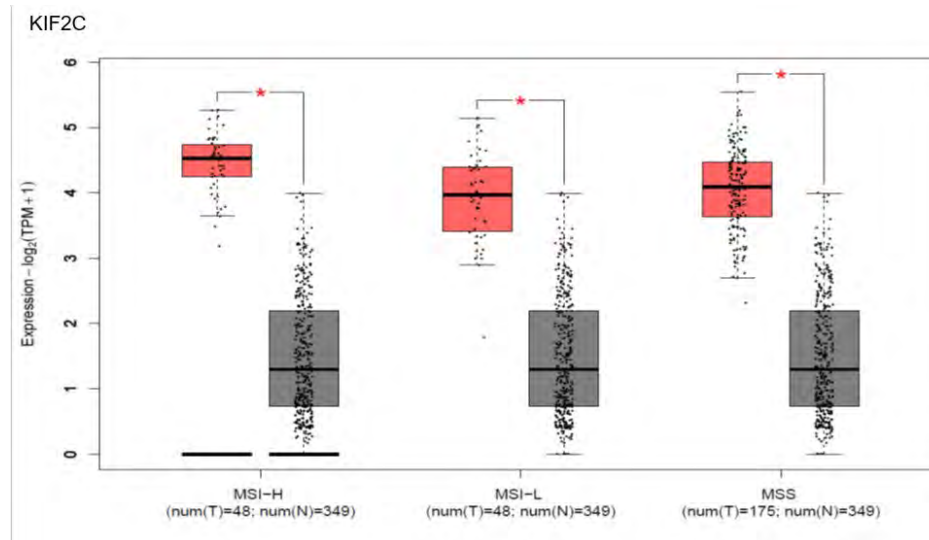


Figure 10: RNA expression data of BIRC5, KIF2C, FOXM1, UBE2C and CDC20 in colon adenocarcinoma (COAD) according to MSI/MSS status, and related normal samples from the TCGA and the GTEx projects (GEPIA database) (TPM= transcript per millions)



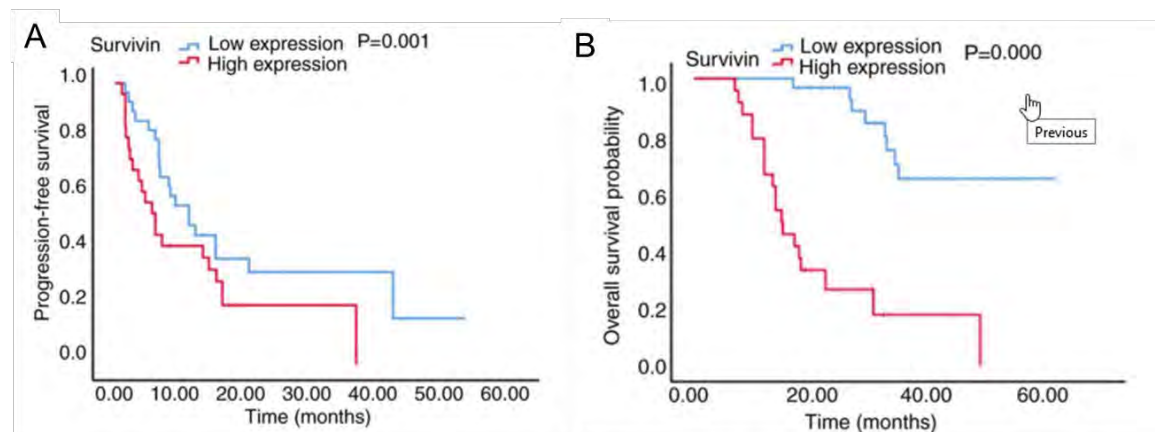




The expression of the selected TAAs is linked to clinical outcome of CRC as exemplified by BIRC5 and FOXM1 data below.

BIRC5 shows no or very low expression in normal colon tissues while BIRC5 is expressed in colon polyps and overexpressed in colonic adenocarcinoma (Jourabchin et al., 2020). Overexpression is observed at both the mRNA and protein levels with overexpression often seen in 80% to 100% of analyzed samples (Adamkov et al., 2015; Hernandez et al., 2011; Jourabchin et al., 2020; Sprenger et al., 2011). Survivin/BIRC5 expression in colon cancer is correlated with the degree of differentiation, depth of invasion, lymph node metastasis and Duke's stage (Huang et al., 2013; Shao et al., 2018), and is associated with poor prognosis (Figure 11) (Gu et al., 2019; Huang et al., 2013).

Figure 11: Kaplan-Meier survival curves for PFS (A) and OS (B) in patients with stage IV colorectal cancer (N=71) (Gu et al., 2019)

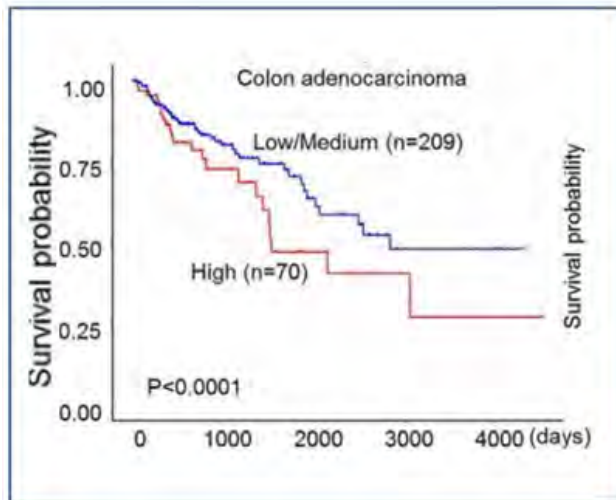


FOXM1 overexpression in colorectal cancer has also been documented in many reports both at the mRNA and protein level, with overexpression observed in between 50 % to 85% of analyzed samples (Chu et al., 2012; Uddin et al., 2011; H. G. Zhang et al., 2016). FOXM1 expression is directly linked to prognosis in colorectal carcinoma and FOXM1 levels correlate with cancer progression, lymph node and liver metastasis, and high TNM stages (Chu et al., 2012; Yao et al., 2018; H. Zhang et al., 2016). The important role of FOXM1 in



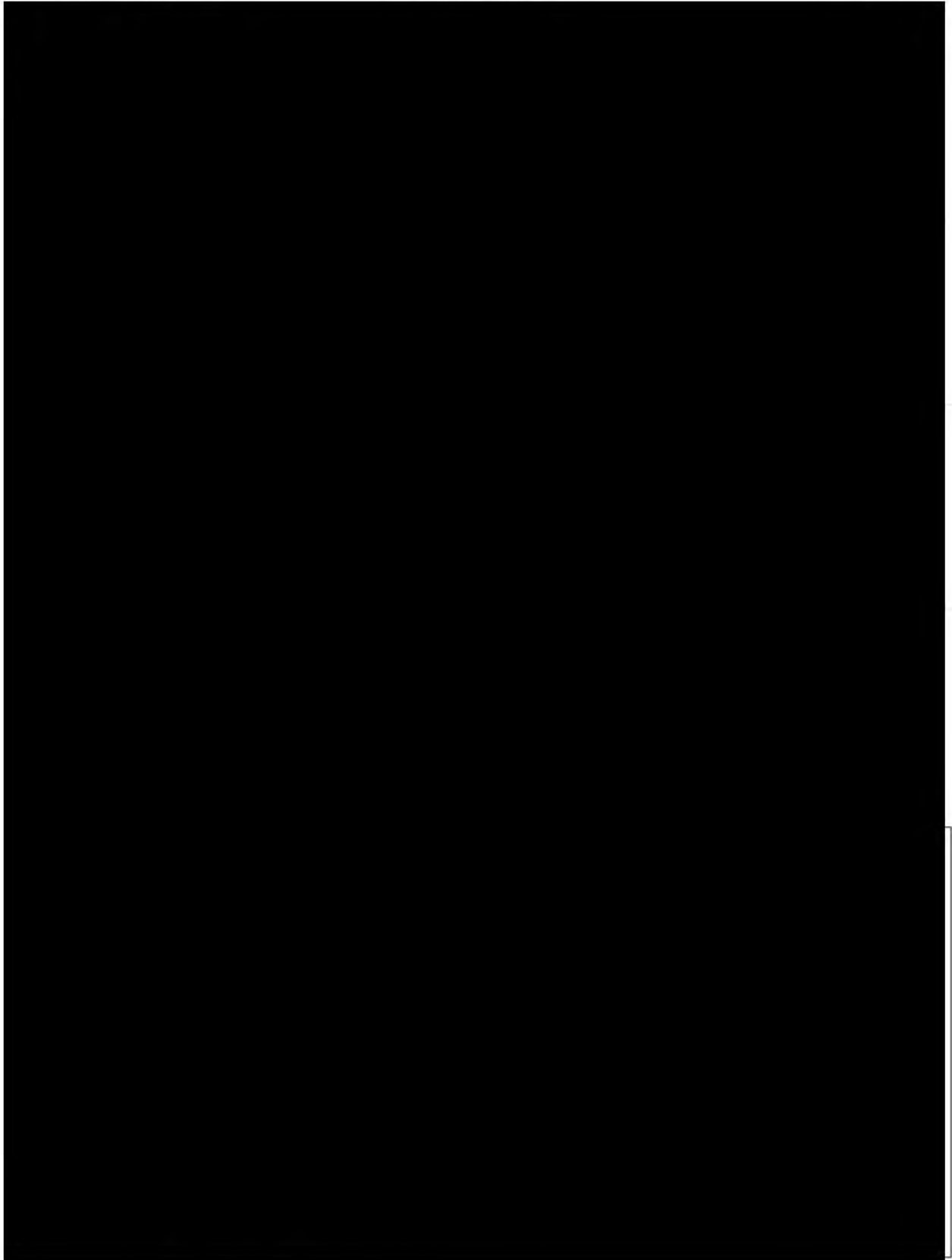
metastasis development in CRC is thought to be linked to induction of the epithelial-mesenchymal transition (Fei et al., 2017; C. Zhang et al., 2016).

Figure 12: FOXM1 gene expression association with overall survival in patients with colon adenocarcinoma; Kaplan–Meier analyses of overall survival according to FOXM1 expression levels in adenocarcinoma patients (Liao et al., 2018)



Overexpression of UBE2C, CDC20 and KIF2C in colorectal cancer and links to clinical outcome have also been documented in a large number of studies. More information is available in the current IB of EO4010.

Of note, peptide specific CD8⁺ T cells can be detected in healthy donors and expanded (Figure 13), and such T cells can kill HLA-A2 cells (T2 cell line) loaded with either the bacterial peptides, or the human counterpart (Figure 14).



1.2.2 UCP2 – Helper peptide

To generate an efficient immune response, the helper peptide UCP2 is included in EO4010. UCP2 was described by Godet ([Godet et al., 2012](#)). 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 (Galaine et al., 2015).

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 (Leão et al., 2018). TERT overexpression achieved via multiple genetic and epigenetic mechanisms could be observed in 80–90% of malignant tumors (Kim et al., 1994; Shay & Bacchetti, 1997).

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 (Abe et al., 2001; Fernández-Marcelo et al., 2016; Liu et al., 2006; Lukman et al., 2000), with the potential to be used as a biomarker and prognostic tool (Bertorelle et al., 2014; Piñol-Felis et al., 2017).

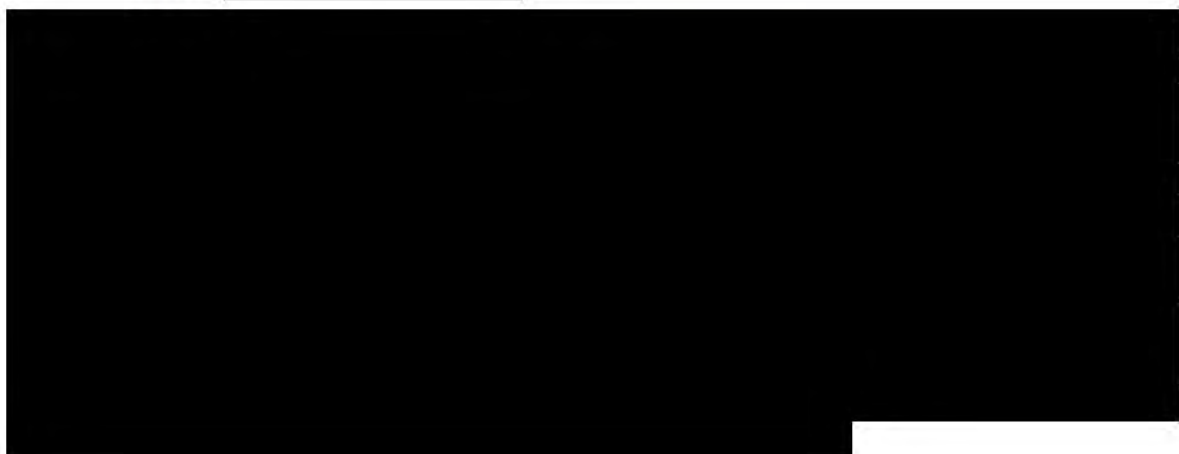
The ability of UCP2 to elicit a specific CD4⁺ T cell responses has been demonstrated in *in vivo* models. Furthermore, spontaneous T cell responses against UCP2 were observed in various types of cancers (Dosset et al., 2012), and this peptide is currently under clinical evaluation in phase 1/2 studies 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 EO4010.

In the EO4010 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 1.2.5.

1.2.3



1.2.4 Combination with anti-PD1 blockade and/or with VEGF/VEGFR blockage

The concomitant administration of EO4010 and an anti-PD1 blocking agent is utilized to optimize the fast expansion of specific T cells without negative feed-back loops, and 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 (Cloughesy et al., 2019; Schalper et al., 2019).

The mechanism of action of anti-PD1 like nivolumab, blocking interaction between PD1 and its ligands, releasing PD1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response aims at achieving a fast expansion of T cells and 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 (Curran & Glisson, 2019; Eggermont et al., 2018; Hellmann et al., 2016), 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 15).

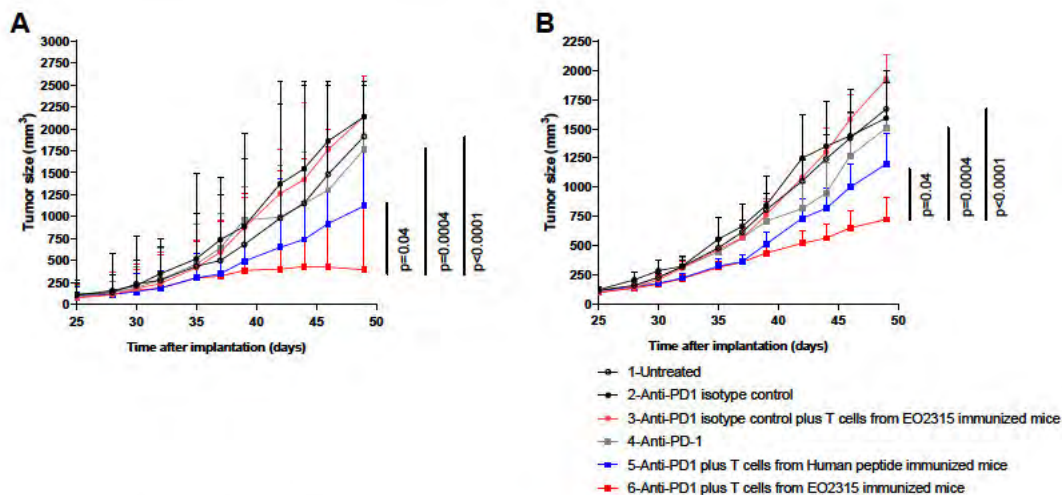
The mechanism of action of Bevacizumab relies on inhibiting the VEGF/VEGFR pathway upon binding to vascular endothelial growth factor A (VEGF-A). VEGF/VEGFR blockade leads to vasculature normalization, permitting increased tumor infiltration of T cells, and activation of effector immune cells through stimulation of the maturation of dendritic cells (DCs) and reduction of the expansion of Tregs and MDSCs (Limagne et al., 2016; Osada et al., 2008; Terme et al., 2013).

Figure 15: Mean tumor growing curves of U87MG glioblastoma engrafted mice following adoptive transfer of T cells from HHD DR3 mice vaccinated with EO2315 or human peptide, w/o combination with anti-PD1 antibody

Note: EO2315 is a microbiome-derived peptide, and proof of concept progenitor part of EO2401.

Untreated mice (1), Anti PD1 isotype control (2), Anti PD1 isotype control plus T-cells from EO2315 immunized mice (3), Anti PD1 (4), Anti PD1 plus T-cells from human peptide immunized mice (5), Anti PD1 plus T-cells from EO2315 immunized mice (6).

A. results expressed as median; B. results expressed as mean plus SEM



1.2.5 Early clinical development of microbiome-derived peptides in solid tumors

EO4010, the compound to be used in the current trial is including two microbiome derived peptides (EO2317 and EO2318), and the helper peptide UCP2, from the first microbiome-derived therapeutic vaccine compound EO2401 which is already investigated in clinical trials.

Thus, EO4010 includes the same components as EO2401, except the microbiome derived peptide EO2316, mimicking an epitope on the TAA interleukin 13 receptor alpha-2 (IL-13R α 2). The reason to exclude peptide EO2316 from compound EO4010 is that IL-13R α 2 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 reason to expand the number of microbiome-derived peptides in EO4010 was to further enhance the potential to overcome possible tumor heterogeneity and reduce tumor escape.

In the following, the compound EO2401, is described regarding early clinical development, especially with regard to safety since it is assumed that the safety profile of EO4010 will be at par with EO2401. Note, [REDACTED], 170 patients with glioblastoma and adrenal tumors had started treatment with EO2401 in combination with nivolumab (and in some patients with glioblastoma also in combination with bevacizumab as a third drug) showing the ability to generate strong systemic immune responses against the targeted TAAs, correlating with clinical outcome, and being well tolerated; the safety profile being consistent with the profile of the combination partner nivolumab (and when applicable bevacizumab), with the only addition of local administration site reactions (most commonly grade 1, erythema, induration, and sometimes pain).

The initial clinical development of EO2401 includes 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 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 [REDACTED]), and expansion cohorts for assessment of further safety and tolerability, immunogenicity, and preliminary efficacy during treatment with EO2401 in combination with nivolumab, and EO2401 in combination with both nivolumab and bevacizumab concomitantly. A total of 100 patients have been treated in this study. and the first patient treated in trial EOGBM1-18 received EO2401 monotherapy [REDACTED].

The second early clinical development trial including EO2401, EOADR1-19, is a multicenter (before expansion to a randomized trial including 10 sites in Denmark, France, Germany, Italy, Netherlands, Spain, Sweden, and USA, and after expansion to a randomized trial including approx. 10 further sites in the same countries plus planned Switzerland and UK), 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 [REDACTED]), 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 first patient treated in trial EOADR1-19 received EO2401 in combination with nivolumab on August 11, 2020. A total of 70 patients have been treated in this study.

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 [REDACTED]. 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.

1.2.5.1 EO2401 clinical safety experience

Six planned safety assessments have been performed to define the early development safety profile of EO2401 in combination with nivolumab +/- bevacizumab:

- EOGBM1-18: a protocol pre-planned interim assessment with a clinical cut-off [REDACTED], a second planned follow-up assessment for external data presentations with a clinical cut-off [REDACTED] and third follow-up assessment with a clinical cut-off 2 [REDACTED]
- EOADR1-19: a planned safety assessment with a database cut-off [REDACTED], a second planned follow-up assessment for external data presentations with a clinical

cut-off 2022-JUL-04 and third follow-up assessment with a clinical cut-off 2023-AUG-21

Summaries and conclusions of the most recent safety assessments from both trials are included below (full reports are on file by the Sponsor).

Of note, based on IDMC assessments, the safety lead-in parts of both trials (EOGBM1-18 and EOADR1-19) have run with a minimum number of patients (each 3 patients) due to lack of safety issues (no safety concern events detected), and all expansion cohorts in both trials have, after further IDMC assessments, been opened as planned for recruitment.

The dose and schedule established for EO2401 based on studies EOGBM-18 and EOADR1-19 and IDMC recommendations, is 300 µg/mL of each of the peptides in the mix per dose administration (*i.e.* 1.2 mg/mL protein per administration with the included 4 peptides), and administration by 4 priming injections SC at 2-weekly intervals, followed by boosting injections starting at 4 weeks after the fourth priming injection; continued boosting injections given on a 4-weekly interval.

The EO2401 dose and schedule per above have been used in combination with nivolumab, and in combination with nivolumab/bevacizumab.

The nivolumab dose and schedule applied in trial EOGBM1-18 followed the early development principles for nivolumab, *i.e.* a dose of 3 mg/kg administered every 2 weeks in combination with EO2401. For trial EOADR1-19 the more convenient, and also labelled, dose/schedule instruction was:

- Nivolumab is to be administered as an IV infusion starting from the first priming injection of EO2401, and then following the schedule in priming and boosting, of EO2401. The nivolumab infusion is to start 3 hours after the EO2401 administration. The dose of nivolumab should be 240 mg every 2 weeks for the first 3 administrations, and from the fourth administration, and onwards, a nivolumab dose of 480 mg every 4 weeks is to be applied.

Note, all patients have received EO2401 by SC administration together with the [REDACTED]

Summary of safety of EO2401 in combination with nivolumab +/- bevacizumab in patients with recurrent glioblastoma

Trial EOGBM1-18 includes Cohort 1 (safety lead-in, 3 patients, plus expansion to test schedule; patients with first recurrent measurable disease; treatment EO2401 for 4 weeks, then switch to EO2401/nivolumab if treatment tolerated), Cohort 2a (patients with first recurrent measurable disease; treatment EO2401/nivolumab), Cohort 2b (patients with non-measurable disease; adjuvant treatment after resection for first recurrent disease; treatment EO2401/nivolumab), and Cohort 3 (patients with first recurrent measurable disease; treatment EO2401/nivolumab/bevacizumab; standard dose bevacizumab 10 mg/kg q2 weekly).

For trial EOGBM1-18 the third assessment includes a total of 100 patients who had started study treatment: 21 in Cohort 1, 38 in Cohort 2a, 6 in Cohort 2b, 9 in Cohort 2c and 26 in Cohort 3. All patients have been treated with the combination of EO2401 (given SC together with the [REDACTED]) and nivolumab if included in Cohorts 1, 2a, and 2b, and with EO2401, nivolumab, and bevacizumab (standard dose 10 mg/kg q2 weekly) in Cohort 3 per protocol EOGBM1-18. The difference for patients treated in Cohort 1 is that they had a 4-week (2 administrations) initial part of study treatment with EO2401 monotherapy, before nivolumab was added to EO2401 (all other cohorts had a simultaneous start of EO2401 and nivolumab on

day 1). After global amendment 2 leading to protocol version 3, 18 patients have been starting treatment in Cohort 1, 15 patients in Cohort 2a, and 3 patients in Cohort 2b (a total of 36 patients), and these patients have had the possibility to receive extended patient management measures including time limited neurologic symptom driven low-dose bevacizumab (5 mg/kg q2 weekly).

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

In conclusion, the combination of EO2401 (administered SC with the [REDACTED] and nivolumab +/- bevacizumab used for treatment of patients with recurrent glioblastoma, is well tolerated

with a safety profile consistent with the safety profile of nivolumab monotherapy, and when applicable the safety profile of bevacizumab monotherapy, except the addition of local administration site reactions.

The safety profile consistency between EO2401/nivolumab +/- bevacizumab and nivolumab monotherapy builds on assessments of trial EOGBM1-18 versus EMA and FDA labelling documents for nivolumab showing:

- consistency of quality and frequency of most common events,*
- consistency of key safety parameters (frequency of AEs leading to treatment discontinuation, frequency of SAEs, frequency of Grade 3/4 AEs), and*
- consistency of findings of immune-mediated events.*

Of note, administration site reactions seen in trial EOGBM1-18 are below, or well within the expected frequency for any antigen administered together with [REDACTED] [REDACTED] van Doorn et al. indicate injection site inflammation, edema and redness, damage, or induration in 40% to 100% of patients in randomized trials; assumed around 70% by SC route in non-randomized trials [REDACTED]

Summary of safety of EO2401 in combination with nivolumab in patients with adrenal tumors

Trial EOADR1-19 includes Cohort 1 (safety lead-in; patients with adrenal tumor and previously treated disease; treatment EO2401/nivolumab), Cohorts 2A/B (patients with adrenocortical carcinoma), and Cohorts 3A/3B (patients with malignant pheochromocytoma/paraganglioma); A, denotes patients with previously treated disease; and B, patients with no previous treatment for advanced/metastatic disease.

For trial EOADR1-19 the third assessment included a total of 65 patients who had started study treatment, including 33 patients with adrenocortical carcinoma (ACC; Cohorts 2A/2B), 14 patients with adrenocortical carcinoma (ACC; randomized extension of Cohort 2A) and 18 patients with malignant pheochromocytoma/paraganglioma (MPP; Cohorts 3A/3B).

Note, all patients have been treated with the combination of EO2401 (given SC together with the [REDACTED]) and nivolumab per protocol EOADR1-19 (with the exception of patients from Cohorts C2A-II and C2A-III). Baseline characteristics:

In the non-randomized part of the study:

[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]	
[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]	
[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]	
[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]	
[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]		[REDACTED]	



In the whole study population, 63% of treated patients had liver metastases (65% ACC/55%

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

In conclusion, the combination of EO2401 (administered SC with [REDACTED]) and nivolumab is well tolerated with a safety profile consistent with the safety profile of nivolumab monotherapy, except the addition of local administration site reactions.

The safety profile consistency between EO2401/nivolumab and nivolumab monotherapy builds on assessments of trial EOADR1-19 versus EMA and FDA labelling documents for nivolumab showing:

- *consistency of quality and frequency of most common events,*
- *consistency of key safety parameters (frequency of AEs leading to treatment discontinuation, frequency of SAEs, frequency of Grade 3/4 AEs), and*
- *consistency of findings of immune-mediated events.*

Of note, administration site reactions seen in trial EOADR1-19 are below, or well within the expected frequency for any antigen administered together with [REDACTED]

Overall conclusion regarding safety of EO2401

Overall, considering 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.

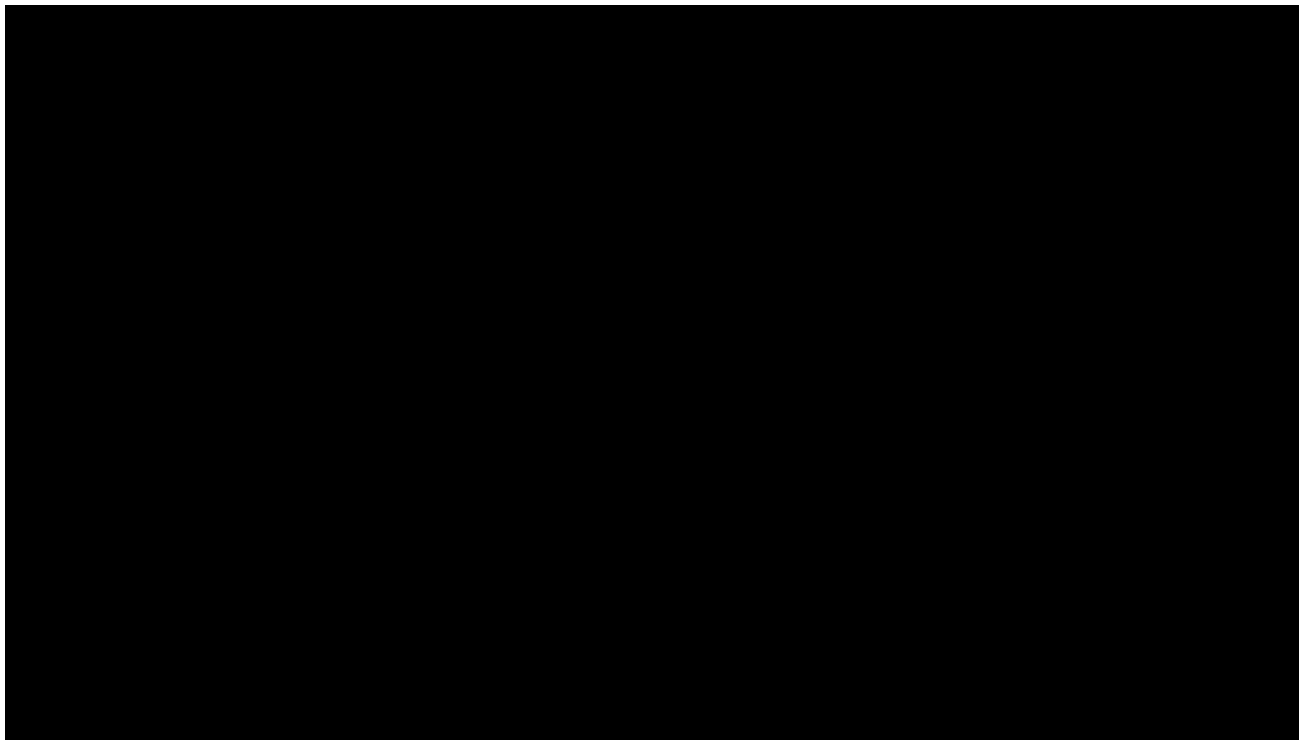


1.2.5.2 EO2401 clinical efficacy and immunogenicity

Clinical efficacy and immunogenicity of trials EOGBM1-18 and EOADR1-19 have been presented at ASCO,ESMO and SNO 2023 ([Baudin et al., 2023](#); [Reardon et al., 2023](#); [Wick et al., 2023](#)) .

Clinical activity has been noted in both trials EOGBM1-18 and EOADR1-19 and is not going to be further summarized here, except for the important findings that:

- Efficacy of EO2401/nivolumab/bevacizumab seems encouraging in a historical perspective, including a median survival of 14.5 months and an 18-months survival rate of 43% in patients with first recurrent glioblastoma. the 24-months survival rate achieved with EO2401/nivolumab in patients with adrenocortical carcinoma (57% 1st line and 31% 2nd/3rd line treatment) is at pair with what has been seen earlier with the most efficacious triplet chemotherapy (etoposide, doxorubicin, cisplatin,) plus mitotane (EDP-M) in a pure 1st line setting. There seems to be an inverse relationship by linear regression between degree of tumor shrinkage and strength of CD8 T cell specific immune response (assayed by tetramers after in vitro stimulation of PBMC; max response during treatment) Figure 16.



Preliminary clinical data collected with EO4010 in current study is provided in the Investigator brochure. No SAE has been reported at the cut-off of IB November 27th 2023.

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

The current trial is a multi-center, open-label, non-comparative, first-in-man phase 1/2 study, to assess safety, tolerability, immunogenicity, and preliminary efficacy of EO4010 in combination with nivolumab in patients with unresectable, previously treated locally advanced or metastatic CRC.



There is lack of therapy options for patients with unresectable, previously treated locally advanced or metastatic CRC after completion of standard of care treatments with an adequate chance to achieve tumor shrinkage, and thus there is a need for new therapeutic approaches.

Application of rationally developed immunotherapies might be one avenue to explore to increase the battery of efficacious treatments for patients with previously treated 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.

EO4010, to be evaluated in the current phase 1/2 clinical trial is a therapeutic peptide vaccine composed of five microbial-derived peptides (EO2317, EO2318, OMP10, OMP11, and OMP12) mimicking cytotoxic T cell (CD8+ T cell) HLA-A2 restricted epitopes from five different TAAs: BIRC5, FOXM1, UBE2C, CDC20, and KIF2C. EO4010 also includes the helper peptide (CD4+ T cell epitope) universal cancer peptide 2 (UCP2), derived from hTERT.

The peptide drug substances are dissolved in an aqueous solvent system (*i.e.* water for injection / DMSO) and then emulsified with the [REDACTED] resulting in a water-in-oil (w/o) emulsion for subcutaneous injection.

For details regarding the non-clinical safety evaluations of the microbiome derived therapeutic vaccine concept, including EO4010, see the current IB of EO4010, 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) ([Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)).

The safety, and benefit/risk, profile of bevacizumab is well known via the multiple labels for different malignant diseases already approved globally, also including patients with metastatic CRC, including very encouraging results (combination TAS 102 with bevacizumab) from SUNLIGHT study (see [Section 1.1.6](#)).

The addition of bevacizumab to the EO4010 and nivolumab combination is supported by the fact that CRC is highly angiogenic and is associated with elevated expression of VEGF, and that VEGF mediates immunosuppression via inhibition of dendritic cell maturation, reduction of T cell tumor infiltration, and promotion of inhibitory cells in the tumor microenvironment. Bevacizumab blockade of VEGF is thus assumed to enhance the

possibilities for EO4010 and/or EO4010 and nivolumab to work. In addition, bevacizumab gives the possibility for patients to have a slightly prolonged time to progression (even if survival is not prolonged) meaning that patients might benefit from a longer time administration of EO4010 and/or EO4010 plus nivolumab.

Three of the six peptides constituting EO4010 (the microbiome-derived peptides EO2317 and EO2318, and the helper peptide UCP2) are included in the compound EO2401 which in combination with nivolumab has been thoroughly investigated in two phase 1/2 trials in 170 patients (by November 2023) with glioblastoma and adrenal tumors, showing the ability to generate strong systemic immune responses against the targeted TAAs correlating with clinical outcome, 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 (administered with the oil-in-water [REDACTED]) in combination with nivolumab, and nivolumab/bevacizumab is described in [Section 1.2.5](#).

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 EO4010, with the addition of local administration site reactions (erythema, induration, and sometimes pain) in around half of the patients and characteristics probably more resembling patients treated in trial EOADR1-19 (see [Section 1.2.5.1](#) above), provided longer treatment durations are achieved.

The location of EO4010 injections is rotating, *i.e.* an injection site will be used every fourth

[REDACTED]

[REDACTED]

[REDACTED]

Beside the above precaution regarding utilization of administration sites for EO4010, guidance regarding how to handle documentation and caretaking of local administration site reactions have been developed based on prior experiences from the compound EO2401; the guidance is outlined in [Section 6.5.3](#).

In addition to above, and as for all administrations of immunogens, it cannot be excluded that immediate type of allergic reactions might occur, even if immediate type allergic reactions at injections of EO2401 has not yet been seen in any patient at any administration of EO2401 during the early clinical development; special precautions and guidance are outlined for this type of potential reactions in [Section 12.4](#) of the protocol.

The selection of antigens targeted by EO4010 and their expression in normal tissues makes it unlikely that other normal cells (*for an extensive description and justification of the use of each of the individual peptides, including lack of normal tissue expression, see the current IB of EO4010*), would be attacked by EO4010 expanded T cells even though target antigens expression can be found in the testis which is an immune privilege organ. The immune privilege of testis is associated with the physical structure, the local active immunosuppressive milieu, and a systemic immune tolerance ([Zhao et al., 2014](#)). In this organ, dendritic cells suppress antigen-specific immunity and T cell activation allowing tolerance of both allo-antigens and immunogenic autoantigens ([Fijak & Meinhardt, 2006](#)).

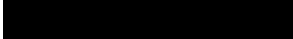


However, provided EO4010 induces an efficacious expansion of T cells specific for the targeted TAAs with ability to kill tumor cells expressing these TAAs, it cannot be ruled out that such antigen specific T cells might also recognize these antigens if expressed, and presented by MHC, at a high enough level (*i.e.* expressed as in tissue from CRC) on non-cancerous cells. Thus, if killing of tumor cells by T cells expanded by EO4010 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 (*Opdivo* | *European Medicines Agency*, 2023; *Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023*, n.d.). 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 EO4010 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 6.5.1](#)). Of note, the early clinical development of the other therapeutic vaccine 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 1.2.5.1](#), (*Opdivo* | *European Medicines Agency*, 2023; *Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023*, n.d.)).

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 EO4010 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 EO4010 administrations at non-acceptable toxicity.

In this trial, as in the early development of EO2401, subcutaneous (SC) administration of EO4010 will be applied based on the general notion that this is a well-accepted, and clinically easy and reproducible way for vaccine administration.

The peptide dose most utilized in non-clinical testing of the components of different microbiome-derived therapeutic vaccines was 100 µg per peptide. However, given that there is no predefined conversion factor to enable extrapolation from a safe dose in animals to a potentially safe starting dose in humans for this type of cancer vaccine product (as referred in the US FDA guidance for industry “Clinical Considerations for Therapeutic Cancer Vaccines”, October 2011 (*Clinical Considerations for Therapeutic Cancer Vaccines* | *FDA*, n.d.)), the clinical dose of EO2401 for the first cohorts in both EOGBM1-18 and EOADR1-19 was chosen based on reported clinical study results with other peptides for which immune responses and efficacy were observed after injection of 300 µg per peptide. Considering the results regarding safety, immunogenicity, and efficacy achieved with EO2401 at the selected dose of 300 µg per peptide in two clinical trials (see [Section 1.2.5](#)), the starting peptide dose for the current trial has also been selected to be 300 µg per peptide.

Immunogenicity data to support the use of the UCP2 helper peptide, combined with the microbiome-derived peptides, together with  Sterile as adjuvant have

been generated *in vivo* in HLA-A2 transgenic mice. The presence of Montanide ISA 51 VG supports the induction of an immune response in mice and the addition of a Th1 helper peptide as UCP2 showed an enhancing effect on T cell response induced by the microbiome-derived peptide EO2315 against itself and the corresponding human TAA (see the current version of the EO4010 IB).

The dosage regimen for EO4010 including a priming schedule was chosen to induce an efficient immune response (), and to avoid exhaustion () break thereafter. The priming period is followed by a boosting period with injections every () 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 EO4010 (see [Section 1.2.5](#)). Of note, based on the experience from the clinical trials EOGBM1-18 and EOADR1-19 a specific dose interval adjustment schedule for EO4010 has been developed in relation to potential appearance of the assumed rarely developing Grade 3 local administration site reactions (see [Section 6.5.3](#)).

Nivolumab route of administration, dosage, dosage regimen, and treatment periods are aligned with current labeling for the compound (*Opdivo* | [European Medicines Agency, 2023](#); *Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023*, n.d.).

Bevacizumab will be administered at the dose of () from 1st administration and onwards every () until disease progression or unacceptable toxicity. This route of administration, dosage, dosage regimen, and treatment periods are aligned with current labeling for the compound (*Zirabev* | [European Medicines Agency, 2023](#); *Zirabev-Drugs@FDA: FDA-Approved Drugs February 2023*, n.d.).

Of note, the interval between nivolumab administrations in the current trial is adjusted according to the administrations of EO4010, *i.e.* with () administrations () followed by () during the boosting period of EO4010 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; but based on clinical practice and recommendations from the authorization holder for nivolumab (Bristol-Myers Squibb) the maximum duration of nivolumab is set to (). For alignment, the maximum duration of study treatment is set to () for all cohorts of this study. However, patients with an ongoing clinical effect at () will be discussed individually between the treating physician and the Sponsor.

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

In addition, the early development of EO2401 indicated the potential of efficacy of the therapeutic vaccine 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, and non-checkpoint favorable disease characteristics, *i.e.* lack of PD-L1 expression in tumor, low tumor mutational burden, and microsatellite-stable disease).

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 EOCRC2-22/AUDREY.



1.3 History of amendments

EOCRC2-22 protocol version (date)	Key points of protocol version (only substantial changes listed)
Version 1.0 (2022-AUG-29)	For authority submissions
Version 1.1 (2023-FEB-07)	French specific version including addition of exclusion criteria for French sites as well as editorial changes
Version 2.0 (2024-JAN-15)	Global amendment n°1, change of Cohort 3 design (3a and 3b) with addition of bevacizumab



2 RATIONALE

2.1 Rationale for the study

Treatment options for patients with metastatic colorectal cancer who have received the long-standing standard treatment options including fluoropyrimidine-, oxaliplatin-, and/or irinotecan-containing chemotherapy regimens, combined, or not, with *e.g.* bevacizumab, cetuximab, panitumumab, depending on tumor characteristics, are sparse.

The available options of regorafenib (Stivarga) and trifluridine/tipiracil (TAS-102; Lonsurf) have in the phase 3 setting achieved, statistically significant, but clinically very limited efficacy changes above what is achieved with placebo, *i.e.* median progression-free survival addition of around 10 days, median survival addition of less than 2 months, and objective response rates less than 2%.

Thus, metastatic CRC already treated with the old classical treatment options of fluoropyrimidine-, oxaliplatin-, and/or irinotecan-containing chemotherapy, continues to be a major therapeutic challenge with a considerable number of patients experiencing premature death due to disease recurrence indicating that novel treatment concepts are needed.

One novel treatment option, with adequate efficacy and acceptable safety, have however become available for a limited subset of patients with CRC; *i.e.* treatment with the anti-PD1 blocking monoclonal antibodies nivolumab and pembrolizumab which are now labelled possibilities for patients with MMR deficient or MSI-high metastatic CRC, a group constituting < 5% of patients with metastatic disease (Biller & Schrag, 2021). Of note, the majority of patients with metastatic CRC have disease characterized by mismatch repair proficiency or microsatellite-stable type, meaning that they do not have appreciable benefit of checkpoint inhibition therapy by itself (Le et al., 2015, 2017).

The current trial will exclude patients who are possible to treat with checkpoint inhibitors according to current labels, *i.e.* patients with MMR deficient or MSI-high metastatic CRC.

However, the trial will build further on the success of immunotherapy in a subset of patients with CRC per above and utilize the microbiome-derived therapeutic vaccine concept in conjunction with anti-PD1 blockade as an innovative option for testing of a rationally developed immunotherapy for CRC with broader utility than checkpoint inhibition by itself.

The microbiome-derived therapeutic vaccine concept as such, including the combination with nivolumab, has already been tested in the clinical setting 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. As described in [Section 1.2.5](#) efficacy has been noted in adrenocortical carcinoma, *i.e.* in a population of patients where the majority have liver metastases, most often combined with other metastatic sites, including lung, and non-checkpoint favorable disease characteristics, *i.e.* lack of PD-L1 expression in tumor, low tumor mutational burden, and microsatellite-stable disease, a situation not so dissimilar from mismatch repair proficient and microsatellite-stable CRC.

One relevant reason for immune resistance leading to low activity of PD-1/PD-L1 blockade in the majority of colorectal tumors might be 

 A peptide-based therapeutic vaccination approach could counteract such a problem aiming at delivering immunogenic peptides corresponding to specific TAAs to patients. The goal would be to target the patient's antigen presenting cells to induce efficient presentation of cancer epitopes to T cells that in turn leads to an efficient sustained immune response against cancer cells expressing the same antigens at the tumor site.

To counteract earlier problems with the antigens used for therapeutic cancer vaccinations, the concept behind the current trial includes an innovative microbiome-based approach for the development of therapeutic peptide cancer vaccines. Numerous peptides mimicking specific TAAs that are overexpressed in CRC have been identified in the human microbiome; such peptides can from an immunological perspective be considered as non-self. Specific peptides, further selected based on multiple parameters including high-affinity binding to MHC class I, have been shown to induce strong immune responses in non-clinical models, not only recognizing the microbiome-derived peptide used for immunization but also the nominal human TAA expressed by tumor cells (*i.e.* cross-reactivity have been shown; see the current version of the EO4010 IB).

Although tumor cell overexpressed TAAs are presented via the MHC class I receptors in the form of short peptide sequences (a prerequisite for the possibility to target the TAAs with T cells for tumor cell killing), these peptides are weak inducers of the immune system as they are self-epitopes for which (auto) reacting T cells (especially the most reacting ones) are naturally depleted through thymic deletion. However, the gut immune system, where around 70% of all T cells reside, is tolerant to the equivalent, non-self, microbiome-derived epitopes (Barbi et al., 2014); such epitopes are included in the microbiome-derived peptides developed to mimic CRC TAAs as part of the compound EO4010. Furthermore, it has been demonstrated that the general human population has generated a memory repertoire of the mentioned tolerized T cells recognizing the peptides from the microbiome (Hegazy et al., 2017). These T cells are surprisingly abundant, can be circulating, and display memory phenotypes, all properties that make them ideal to be targeted in a therapeutic vaccination protocol. The tolerance of the T cells can be overcome by repeated antigen challenges together with an adjuvant, which is the immunization principle proposed for the current trial (for supportive data of the use of adjuvant and a helper peptide at immunizations see the current version of the EO4010 IB).

Thus, the strategy behind the current trial is to identify and then use microbiome-derived peptides to reactivate their associated memory T cells, where the peptides are almost identical to portions of known TAAs (the microbiome-derived peptides are mimicking the known TAAs). The mimicking peptides will activate memory T cells that will cross-react with the nominal TAAs, thereby enabling an attack against the tumor cells.

Building on the already known mechanisms of action of anti-PD-1/PD-L1 blockade there is a strong support for the combination of EO4010 and such blockade, which in addition is supported by pre-clinical data obtained in a tumoral model (see Section 1.2.4 and the current version of the EO4010 IB).

Amendment 1 introduces Cohort 3 (3a and 3b) initiation. In this cohort 10 patients will be treated in combination EO4010 with nivolumab and bevacizumab (3a arm) and 5 patients will be treated in combination EO4010 and bevacizumab (3b arm). Two arms will be conducted in parallel to initiate an evaluation of nivolumab role in this combination.

Bevacizumab binds to VEGF, the key driver of vasculogenesis, and thereby inhibits the binding of VEGF to its receptors (VEGFR). Flt-1 (VEGFR-1) and KDR (VEGFR-2) on the surface of endothelial cells. Neutralizing the biological activity of VEGF regresses the vascularization of tumors, normalizes remaining tumor vasculature, and inhibits the formation of new tumor vasculature, thereby inhibiting tumor growth.



Bevacizumab demonstrated significant betterment in association with FU-5 based chemotherapy and in association with TAS-102 in patients with mCRC.

Combination of EO2401 with nivolumab and bevacizumab tested in EOGBM1-18, demonstrated a satisfactory safety profile (i.e. expected profile and absence of emerging safety concerns), fast and strong induction of cytotoxic T cells targeted against tumor cells, and early signs of interesting clinical activity, serves as a rational for this amendment as well.

In conclusion, novel therapeutic approaches are needed to enhance the treatment outcomes for patients with metastatic colorectal carcinoma. An innovative option which is available to test in the clinic, and proposed with the current trial, is the above-described microbiome-derived peptide therapeutic cancer vaccine approach, EO4010, in combination with anit-PD1 blockade and/or with bevacizumab



3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Objectives

3.1.1 Primary objective

The primary objective of this trial is to evaluate safety and tolerability of EO4010 in combination with nivolumab and/or with bevacizumab in patients with unresectable, previously treated locally advanced or metastatic CRC.

3.1.2 Secondary objectives

To assess the following items at therapy with EO4010 in combination with nivolumab and/or bevacizumab:

1. Immunogenicity in relation to T cells of EO2317, EO2318, OMP10, OMP11, OMP12, and UCP2 that compose EO4010; T cell cross-reactivity with the human TAAs FOXM1, BIRC5/survivin, UBE2C, CDC20, and KIF2C will also be evaluated,
2. objective response rate (ORR), disease control rate (DCR), time to response (TTR), and duration of response (DOR),
3. progression-free survival (PFS) and PFS rate at 4 months, and
4. overall survival (OS).

3.1.3 Exploratory objectives

1. To assess expression in tumor tissue (which might be from the primary diagnosis/surgery of CRC, or a later timepoint before study start; or samples from surgeries necessary according to standard of care principles during study participation) of TAAs and immune response/resistance related markers.
2. To assess shifts in peripheral blood, serum, and tumor tissue (if tissue would also become available during study treatment) cell population/marker expressions as for instance specific T cell subpopulations, and myeloid-derived suppressor cells (MDSCs).

3. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

3.2 Endpoints

3.2.1 Primary endpoint

The primary endpoint includes safety and tolerability of EO4010 in combination with nivolumab and /or with bevacizumab 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.

3.2.2 Secondary endpoints

1. 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 EO2317, EO2318, OMP10, OMP11, OMP12, and UCP2 that compose EO4010 by interferon-gamma (IFN- γ) enzyme-linked immunospot (ELISpot), and by intracellular cytokines staining, or multimers staining assays. Cross reactivities with the human TAAs FOXM1, BIRC5/survivin, UBE2C, CDC20, and KIF2C will also be evaluated by the same methods.
2. ORR, DCR, TTR, and DOR as described by RECIST 1.1 and iRECIST criteria.
3. PFS as described by RECIST 1.1 and iRECIST criteria, defined as the time interval from the date of first study treatment administration to the date of progression (by RECIST 1.1 or iRECIST criteria), or death due to any cause, whichever is earlier. Patients without progression or death are to be censored at the time of the last tumor assessment. The PFS rate at 4 months as assessed from the PFS survival curve.
4. OS defined as the time interval from the date of first study treatment administration to the date of death due to any cause. Patients alive will be censored at the date of the last documented follow-up.

3.2.3 Exploratory endpoints

1. Expression by immunohistochemistry (IHC) and/or mRNA in tumor (and normal, if available) tissue of BIRC5/survivin, FOXM1, UBE2C, CDC20, KIF2C and immune response/resistance related markers (e.g. HLA-A2, PD-L1, CD3, CD4, CD8, granzyme, Ki67, [REDACTED]), aiming at defining immune profiles of tumor-infiltrating lymphocytes, and expression of immune markers on tumor cells and in the tumor microenvironment via determination of [REDACTED]. In addition, and as possible by available material, molecular markers (presence, expression levels) will also be determined, including but not limited to mutations, deletions, and/or amplifications, and necessary assessments for determination of cancer molecular subtypes.
2. Percentage of patients with changes on treatment versus baseline in peripheral blood, serum, and tumor tissue cell populations/markers as specific T cell subpopulations, myeloid-derived suppressor cells (MDSCs), and changes in immune related markers as PD-L1/L2 expression.
3. [REDACTED]



[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

4 STUDY DESIGN

4.1 Overall trial design

This is an open-label, multicenter, FIH, phase 1/2 trial to assess safety, tolerability, immunogenicity, and preliminary efficacy of the microbial-derived therapeutic vaccine EO4010 in combination with nivolumab and/or with bevacizumab for treatment of patients with unresectable, previously treated, metastatic CRC.

The schematic trial and cohort designs are presented in **Figure 17** and **Figure 18**.

Figure 17: Schematic trial design

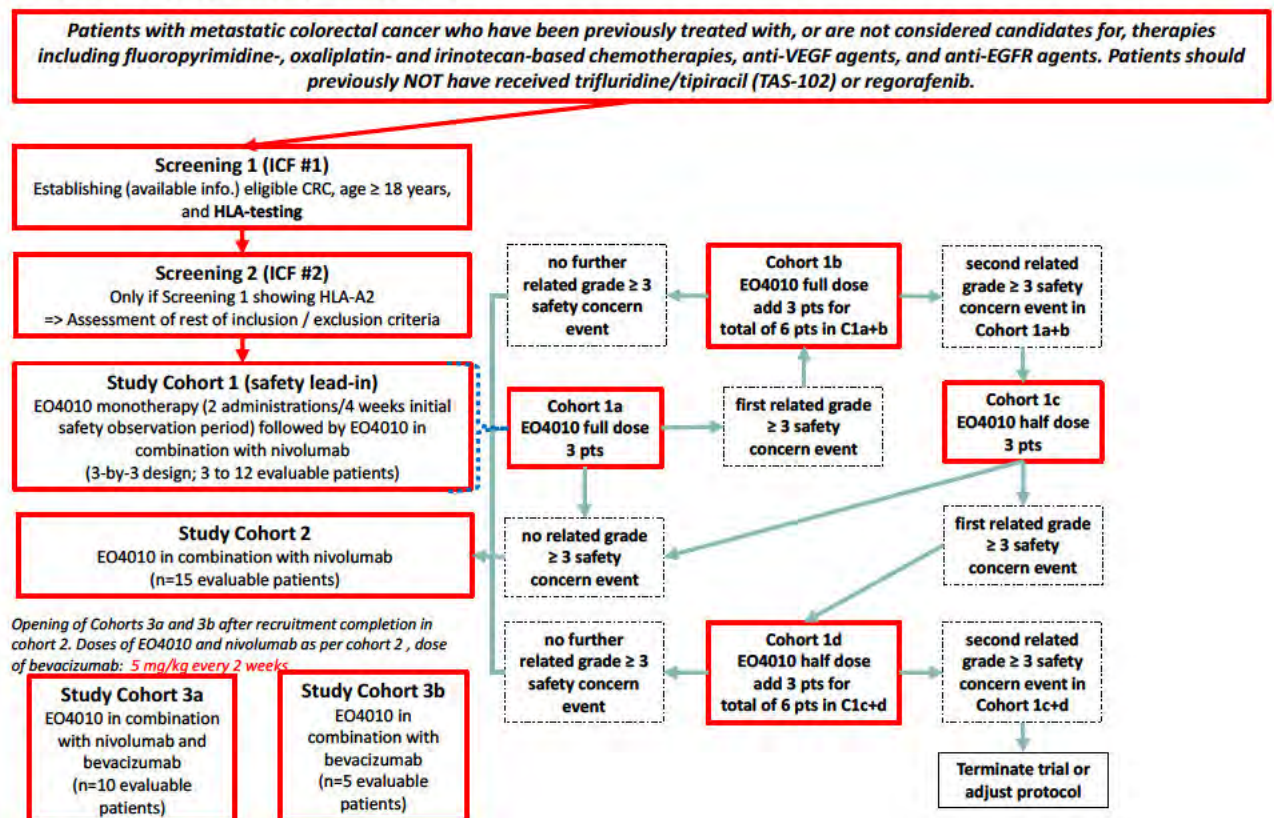


Figure 18: Schematic cohort design



The trial is a 3-cohort study intended to recruit a maximum of 42 evaluable patients in total:

- Cohort 1 includes a safety evaluation by a [REDACTED] of EO4010 monotherapy [REDACTED] of EO4010 each followed by [REDACTED] (observation period) planned to be followed in the same individual patients by continued EO4010 in combination with nivolumab (latter at standard doses per registrations in other settings); 3 to 12 evaluable patients will be included depending on the safety profile of the administered treatments.
- Cohort 2 includes an evaluation of EO4010 at the recommended dose found in Cohort 1 in combination with nivolumab in 15 evaluable patients with CRC.
- Cohort 3 will be run once Cohort 2 recruitment is completed. Cohort 3a includes an evaluation of EO4010 in combination with nivolumab and bevacizumab in 10 evaluable patients with CRC. Cohort 3b includes an evaluation of EO4010 in combination with bevacizumab in 5 evaluable patients with CRC.

Generally, patients evaluable for safety will include all patients who have received at least one dose of EO4010. Patients who are not considered evaluable for safety and did not receive any dose of EO4010 will be replaced (reasons for non-evaluability and pre-treatment safety will be reported). In addition, in Cohort 1, if for reasons other than safety a patient does not receive [REDACTED] and complete the [REDACTED] assessment, the patient will not be counted towards the [REDACTED] and an additional patient should be enrolled (reasons for not being counted towards the [REDACTED] will be reported, as well as applicable safety findings).

There will be a 2-stage consent procedure for the trial, including a first consent for 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 will not match the HLA-A2 prerequisite to receive the HLA-A2 specific EO4010 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 has eligible CRC, and 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 all other aspects of the trial, including procedures.

Eligible patients who meet the inclusion criteria and do not meet any of the exclusion criteria and have provided informed consent per above will be enrolled in the study. The procedures for cohort management, enrollment (approval of start of screening), and approval of start of study treatment are outlined in a study specific Charter.

Each patient will participate in the study up to the end of the study (as defined in [section 4.3.1](#)).

Study treatments will be administered until confirmed tumor progression (iRECIST criteria for confirmed tumor progression are to be used), intolerable toxicity, death, reached maximum individual patient study treatment duration (), patient or Investigator decision, or planned or early termination of the study. At the time of stopping study treatment, appropriate other treatment will be advised by the Investigator on an individual patient basis. The patient should continue 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.

Patients with ongoing clinical benefit on study treatment at the study closure or reached maximum individual patient study treatment duration () will be discussed individually by the treating physician and the Sponsor to find an appropriate way (assumed to be country specific) to manage the patient's further treatment.

All assessments of response and progression in the trial will be determined at the sites by the principal investigators, or designees, according to the RECIST 1.1, and the iRECIST criteria.

Of special note, unlike RECIST 1.1, the iRECIST criteria requires the confirmation of progression and uses the terms iUPD (unconfirmed progression) and iCPD (confirmed progression). Confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks after iUPD. Thus, in the current trial, provided that the individual patient is tolerating the administered treatment adequately, it is allowed continuing trial treatment beyond progression as assessed by the RECIST 1.1. criteria for evaluation of progression according to the iRECIST criteria. Provided good tolerability of the trial treatment and lack of new important symptoms indicating clinical tumor progression the confirmatory scans might be obtained at the current trials planned 8 weekly tumor assessment timepoints.

In the context of confirmation of PD per iRECIST, and the timeline of confirmatory scans to be performed at least 4 weeks, but no longer than 8 weeks after iUPD, it should be specifically noted that in case of any signs of rapidly progressing disease establishment of PD and a following therapy switch should be made also earlier than 4-8 weeks after iUPD, when appropriate.

4.2 Safety oversight, cohorts, and treatments

The safety oversight of the trial is a key design item and 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 specific setup, in addition to standard clinical trial continuous monitoring of adverse events (AEs) and serious adverse events (SAEs), of the comprehensive safety measures of the trial are outlined in the protocol, and include:

- general safety rules,
- definition of safety concern events (the term used in this trial as opposed to the term "dose limiting toxicity" since it is assumed that events in question, *i.e.* needing more rigorous assessment, and potentially leading to adjustments of treatment dosing/scheduling, might not always be dose related in the setting of a compound for therapeutic vaccination as EO4010), including specifics for hematologic, non-hematologic, and hepatic non-hematologic events,
- specification of actions/implications of safety concern events by cohort, and
- specific extended guidance regarding the safety lead-in, dose-finding design, Cohort 1.

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

4.2.1 General safety rules

For all patients, in all cohorts (there are in addition specific rules for Cohort 1; see further below), during the whole trial treatment, the following safety rules apply:

- *All related Grade ≥ 3 adverse events should be reported within 24 hours to the Sponsor via the Medical Monitor (reporting can be via the medical monitoring e-mail [REDACTED] or the medical monitoring telephone [REDACTED] and be reported as an SAE if it meets such criteria. Note, if the eCRF is filled out with the related Grade ≥ 3 adverse events within 24 hours this is also accepted as information to the Sponsor since there will be a special alert system implemented for such recordings in the eCRF.*
- In case a related Grade 5 AE would be observed, recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation regarding further study conduct actions (during the period of assessment and decision making, other already started patients are allowed to continue treatment if no other recommendation is provided by the IDMC and/or decided by the Sponsor).
- In case a related clinical Grade 4 AE would be observed, all components of study treatment will be permanently stopped in the individual patient experiencing the event, and new patient trial treatments will be held until a decision is made by the Sponsor following an IDMC recommendation regarding further study conduct actions (during the period of assessment and decision making, other already started patients are allowed to continue treatment if no other recommendation is provided by the IDMC and/or decided by the Sponsor).
- In case a related clinical Grade 3 AE would be observed, the Investigator will temporarily hold the administration of all components of study treatment in the individual patient experiencing the event until a thorough assessment of the case has been made. A decision



to restart the study treatment in the individual patient experiencing the event can be made by the Investigator responsible for the patient and the Sponsor in collaboration, with the option for any of them to defer the assessment for recommendation of a decision to the IDMC. Other patients in the trial will continue their study treatments as planned, if not otherwise recommended by the IDMC and/or decided by the Sponsor.

- In case of immune-related adverse events or local administration site reactions (LASR) related to study treatment, instructions for treatment modifications as outlined in Protocol [Section 6.5](#) should be followed. Protocol [Section 12.6](#) also includes management algorithms regarding nivolumab and bevacizumab in case of specific adverse reactions.
- See Protocol [Section 12.4](#) regarding precautions in relation to possible infusion related reactions, and treatment of such reactions.
- In addition, any Investigator in the trial or the Sponsor can at the detection of a medically important event (for example grade 2 myocarditis or grade 2 pneumonitis) call upon the IDMC to assess the event and make a recommendation regarding further study conduct actions to the Sponsor for decision.
- Note: Clinical AEs like, for instance, alopecia and transient laboratory abnormalities without clinical significance (*e.g.* no need for medical actions or treatments) will not be considered for actions and decisions per above. The clinical significance of such clinical AEs and laboratory abnormalities will be assessed on a case-by-case basis by the Investigator in collaboration with the Medical Monitor of the Sponsor, and when deemed appropriate in consultation with the IDMC. All decisions by Investigator/Sponsor to consider a related $\geq$ Grade 3 clinical AE or transient laboratory abnormality to be non-clinically significant per above will be documented and the documentation provided in writing to the IDMC, as soon as the assessment by the Investigator and Sponsor is finalized; it will be assessed by the individual IDMC members when received, and it is to be discussed by the IDMC at their next scheduled meeting.
- 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 in the European SmPC or the US PI (*Opdivo* | *European Medicines Agency, 2023; Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.*). The dose modifications for bevacizumab should be taken in accordance with (*Zirabev* | *European Medicines Agency, 2023; Zirabev-Drugs@FDA: FDA-Approved Drugs February 2023, n.d.*)
- The dose modifications for EO4010 follow the same principles as for nivolumab, *i.e.* possible dose modifications include withholding of doses, and treatment discontinuation, except in the potential transition from sub-cohort 1b to sub-cohort 1c in relation to safety events (when a dose decrease is planned; see further below).
- In instances where the label for nivolumab and/or bevacizumab prescribe that the drugs should be withheld or discontinued due to safety events, there is an option to continue the remaining component(s) of the combination (*i.e.*, either EO4010 alone or EO4010 in combination with nivolumab or bevacizumab, respectively) provided such a continuation, after discussion between the treating physician and the Sponsor would be supported. If EO4010 would be withheld or discontinued, nivolumab and bevacizumab will also be withheld or discontinued. In case of discontinuation, the Investigator responsible for the patient in question will advise on adequate further treatment options.



4.2.2 Definitions of safety concern events

The term "safety concern event" has been chosen for the current trial as opposed to the term "dose limiting toxicity" since it is assumed that events in question (*i.e.* needing more rigorous assessment, and potentially leading to adjustments of treatment dosing/scheduling) might not always be dose related in the setting of a compound for therapeutic vaccination (as EO4010).

In patients who experience an AE that meet the criteria to be a safety concern event, administration of study drug(s) should be held (or when specified below, stopped) pending management/resolution of the event, and final assessment of the event (including the further general trial conduct, and individual patient treatment, guidance) as applicable by the IDMC and the Sponsor (see further below). If nothing else stated below, or assessed by the IDMC or Sponsor, treatment, if only temporarily held and not permanently stopped, might be restarted if resolved to the patient baseline, or $\leq$ Grade 1.

General definition by grade (NCI-CTCAE v5.0 grading system) of safety concern events

- *Note, see also below regarding exclusions and definitions regarding specific hematologic safety concern events, non-hematologic safety concern events, hepatic non-hematologic safety concern events, and safety concern events in relation to laboratory parameters.*
- *Note, "related" in the below definitions of safety concern events refer to the causality classification of "suspected", meaning that it is plausible that the AE is caused by the medication(s); i.e., there is a reasonable possibility of a causal relationship. In addition, further guidance regarding applicable definitions can be found in Guideline for Industry, Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH-E2A, March 1995.*
- All related Grade 5 AEs are defined as safety concern events, irrespective of when, or in which cohort, they occur during the trial.
- Related Grade 3 or 4 AEs during treatment with EO4010 monotherapy are defined as safety concern events.
- Related Grade 4 AEs at time periods of combination therapy (*i.e.* during combination treatment with EO4010 and nivolumab and/or with bevacizumab) are defined as safety concern events.
- Related Grade 3 AEs at time periods of combination therapy (*i.e.* during combination treatment with EO4010 and nivolumab and/or with bevacizumab) might also be classified as safety concern events after assessment of the IDMC.
 - *Note, the IDMC might consider any event, regardless of contribution assigned by the Investigator, as a safety concern event.*

Specific definitions (and exclusions) for safety concern events by type; hematologic, non-hematologic, and hepatic non-hematologic events

Hematologic safety concern events are defined as follows:

- Any related Grade 4 hematologic event not further specified in the below list.
- Any Grade 4 neutropenia ($< 0.5 \times 10^9/L$), or Grade 3 neutropenia ($< 1.0 - 0.5 \times 10^9/L$) sustained for 7 or more days or associated with fever ($38.5^\circ C$); complete blood counts should be monitored every 7 days, and growth factor use is permitted.
- In addition, for patients on monotherapy EO4010, any related Grade 3 hematologic event.

Non-hematological safety concern events are defined as follows:

- Any related Grade 4 non-hematologic event.
- In addition, for patients on monotherapy EO4010, any related Grade 3 non-hematologic event, with the following exceptions:
 - Grade 3 inflammatory reaction (e.g. with associated pain, swelling, etc.) attributed to a local antitumor response (e.g. inflammatory reaction at sites of metastatic disease, lymph nodes, etc.) that resolves to $\leq$ Grade 1, or baseline within 72 hours.
 - Grade 3 fever ($>40.0\text{ }^{\circ}\text{C}$ [$>104.0\text{ }^{\circ}\text{F}$] for ≤ 24 hours) not associated with hemodynamic compromise.
 - Grade 3 fatigue that lasts < 72 hours.
 - Grade 3 skin toxicity, including injection site events, that resolves to $\leq$ Grade 1 or baseline within 72 hours with supportive care.
 - Grade 3 gastrointestinal AEs of nausea, vomiting, constipation, abdominal pain, cramping, or dyspepsia that resolves with symptomatic management within 48 hours.
- Related Grade 3 non-hematologic events during combination therapy (i.e. during treatment with EO4010 and nivolumab and/or bevacizumab) might also be classified as safety concern events after assessment of the IDMC.
- Note, related Grade 2 non-hematologic events that are prolonged inordinately, might also be classified as safety concern events after assessment of the IDMC.

Hepatic Non-hematological safety concern events are defined as follows:

- Any related Grade 4 hepatic non-hematologic event.
- Grade 3 elevations in transaminases irrespective of duration.
- Grade 3 elevations in bilirubin irrespective of duration.
- Any event meeting the criteria for Hy's law as follows (all three features):
 - AST and/or ALT $> 3 \times$ ULN,
 - concurrent elevation of total bilirubin $> 2 \times$ ULN without initial evidence of cholestasis, and no alternative etiology can be identified.

4.2.3 Cohort 1 (safety lead-in) treatment and cohort-specific safety rules

Cohort 1 will utilize a safety lead-in [REDACTED] to assess safety and tolerability of EO4010 monotherapy for [REDACTED], to be followed in the same individual patients by continued EO4010 in combination with nivolumab [REDACTED], and thereafter EO4010/nivolumab on a [REDACTED]; nivolumab will be given at standard doses per registrations in other settings). The initial safety evaluation period will include the first [REDACTED] of treatment and be finished in an individual patient by a safety assessment at the week [REDACTED] the visit which is held for the administration of the third dose of EO4010, and the first dose of nivolumab). Patients who are evaluable at [REDACTED], and who do not fulfill any criteria to stop study treatment, will continue study treatment with EO4010/nivolumab according to the Study Schedule (see Protocol [Section 7.1](#)), and be followed for longer term safety and tolerability according to the general safety rules for the trial.

Priming injections of EO4010 will be started at the latest within [REDACTED] of the baseline tumor assessment. The initial dosing regimen will be [REDACTED] administered SC at [REDACTED] intervals, followed by [REDACTED] boosting injections of EO4010 starting at [REDACTED] after the fourth priming injection [REDACTED]. In sub-cohorts 1a and 1b, the full dose of EO4010 (300µg of each peptide, *i.e.* 1 mL of EO4010 in adjuvant) will be administered, and if implemented, the sub-cohorts 1c and 1d will include half the dose of EO4010 (150 µg of each peptide, *i.e.* 0.5 mL of EO4010 in adjuvant).

Nivolumab will be administered as an intravenous (IV) infusion in combination with EO4010, starting from the third priming injection of EO4010. The nivolumab infusion is to start 2 hours after the EO4010 administration. The first dose of nivolumab to be administered at [REDACTED] should be 240 mg, and from the next administration [REDACTED] (V [REDACTED] a nivolumab dose of 480 mg [REDACTED] is to be applied (the dose of nivolumab is then consistent with the longer administration interval).

In Cohort 1, the patients' recruitment will be conducted in accordance with the following rules:

- Patients who are not considered evaluable for safety, *i.e.* did not receive any dose of EO4010, will be replaced (reasons for non-evaluability and pre-treatment safety will be reported).
- If for reasons other than safety, a patient in any of the sub-cohorts of Cohort 1 does not receive [REDACTED] and complete the safety assessment at the start of [REDACTED] should be enrolled (reasons for not being counted towards [REDACTED] will be reported, as well as applicable safety findings).
- All patients will be observed [REDACTED] after an EO4010 administration, and according to standard site practice after nivolumab administration (nivolumab administration will start [REDACTED] after the EO4010 administration).
- The second patient of sub-cohort 1a will not start treatment until the first patient of the cohort has received the second administration of EO4010 (*i.e.* a [REDACTED] recruitment gap between the first and the second study patients). The third patient of sub-cohort 1a will not start treatment until [REDACTED] after the second patient received his/her first administration of EO4010 (*i.e.* a [REDACTED] recruitment gap between the second and the third study patients). The same interval between patients will be applied for sub-cohorts 1b, 1c, and 1d, if they would be implemented.
- During periods of safety assessment and decision making, by the IDMC and/or Sponsor, in relation to a safety event in an individual patient, other already started patients are allowed to continue treatment if no other recommendation is provided by the IDMC and/or decided by the Sponsor (even if new recruitment is stipulated to be held by the safety rules).
- **Sub-cohort 1a (full dose of EO4010) is planned to include 3 evaluable patients:**
 - If in 3 evaluable patients no related Grade ≥ 3 AE (related Grade 3 AEs deemed not to constitute safety concerns by the IDMC are acceptable; see further below) occur during the initial [REDACTED] in the individual patients, the IDMC will assess the totality of available safety data and recommend for decision by the Sponsor further recruitment steps (assumed would be opening recruitment of Cohort 2).



- In case a related Grade 5 AE would occur in any of the three patients, recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation regarding further study conduct actions. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be expanding recruitment by opening sub-cohort 1b for inclusion of 3 patients; a total of 3 patients should also still be included in sub-cohort 1a).
- If a related Grade 4 AE would occur in any of the 3 patients, EO4010 will be permanently stopped in the individual patient experiencing the event, and recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be expanding recruitment by opening sub-cohort 1b for inclusion of 3 patients; a total of 3 patients should also still be included in sub-cohort 1a).
- If a related Grade 3 AE would occur in any of the 3 patients, EO4010 administration will be temporarily held in the individual patient experiencing the event, and recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision made by the Sponsor for the further treatment plan for the individual patient experiencing the event and further recruitment steps (the event might be deemed by the IDMC not to constitute a safety concern in which case the patient can continue treatment and recruitment to the sub cohort 1a continue per plan without an extension of recruitment by opening sub-cohort 1b; alternatively, the IDMC might deem the event to constitute a safety concern in which case EO4010 will be permanently stopped in the patient experiencing the event and recruitment extended by opening sub-cohort 1b for inclusion of 3 patients; a total of 3 patients should also still be included in sub-cohort 1a).
- **Sub-cohort 1b (full dose of EO4010), if opened, is planned to include 3 evaluable patients:**
 - If in 6 evaluable patients in total in sub-cohorts 1a + 1b, only 1 related Grade $\geq$ 3 AE (additional related Grade 3 AEs deemed not to constitute safety concerns by the IDMC are acceptable; see further above and below) occurred during the initial [REDACTED] in the individual patients, the IDMC will assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be opening recruitment of Cohort 2).
 - In case a related Grade 5 AE would occur in any of the patients recruited after the opening of sub-cohort 1b (*i.e.* any of the patients to fill sub-cohort 1a to a total of 3 patients or any of the 3 patients in sub-cohort 1b), recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation regarding further study conduct actions. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be expanding recruitment by opening sub-cohort 1c, at a lower dose level of EO4010, for inclusion of 3 patients).



- If a related Grade 4 AE would occur in any of the patients recruited after the opening of sub-cohort 1b (*i.e.* any of the patients to fill sub-cohort 1a to a total of 3 patients or any of the 3 patients in sub-cohort 1b), EO4010 will be permanently stopped in the individual patient experiencing the event, and recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be expanding recruitment by opening sub-cohort 1c, at a lower dose level of EO4010, for inclusion of 3 patients).
 - If a related Grade 3 AE would occur in any of the patients recruited after the opening of sub-cohort 1b (*i.e.* any of the patients to fill sub-cohort 1a to a total of 3 patients or any of the 3 patients in sub-cohort 1b), EO4010 administration will be temporarily held in the individual patient experiencing the event, and recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further treatment plan for the individual patient experiencing the event and further recruitment steps (the event might be deemed by the IDMC not to constitute a safety concern in which case the patient can continue treatment and recruitment continue per plan without an extension of recruitment by opening sub-cohort 1c; alternatively, the IDMC might deem the event to constitute a safety concern in which case EO4010 will be permanently stopped in the patient experiencing the event and recruitment extended by opening sub-cohort 1c, at a lower dose level of EO4010, for inclusion of 3 patients).
 - After the second related Grade 3 (deemed as a safety concern by the IDMC) or related Grade 4/5 AE, no further patients should be recruited to sub-cohorts 1a or 1b, already started patients are allowed to continue their initiated treatment without any dose modification provided the individual patient is tolerating the treatment well as judged by the treating physician, and if no other recommendation is provided by the IDMC and/or decided by the Sponsor. If any doubt regarding tolerability, the dose should however be adjusted down to the level applied in sub-cohort 1c, for applicable patients from sub-cohorts 1a/b.
- **Sub-cohort 1c (half dose of EO4010), if opened, is planned to include 3 evaluable patients:**
 - If in 3 evaluable patients no related Grade ≥ 3 AE (related Grade 3 AEs deemed not to constitute safety concerns by the IDMC are acceptable; see further above and below) occur during the initial 6 weeks in the individual patients, the IDMC will assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be opening recruitment of Cohort 2; *note, that Cohorts 2, and 3, would include the lowered EO4010 dose*).
 - In case a related Grade 5 AE would occur in any of the three patients, recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation regarding further study conduct actions. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be



expanding recruitment by opening sub-cohort 1d for inclusion of 3 patients; a total of 3 patients should also still be included in sub-cohort 1c).

- If a related Grade 4 AE would occur in any of the 3 patients, EO4010 will be permanently stopped in the individual patient experiencing the event, and recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be expanding recruitment by opening sub-cohort 1d for inclusion of 3 patients; a total of 3 patients should also still be included in sub-cohort 1c).
- If a related Grade 3 AE would occur in any of the 3 patients, EO4010 administration will be temporarily held in the individual patient experiencing the event, and recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further treatment plan for the individual patient experiencing the event and further recruitment steps (the event might be deemed by the IDMC not to constitute a safety concern in which case the patient can continue treatment and recruitment to the sub cohort 1c continue per plan without an extension of recruitment by opening sub-cohort 1d; alternatively, the IDMC might deem the event to constitute a safety concern in which case EO4010 will be permanently stopped in the patient experiencing the event and recruitment extended by opening sub-cohort 1d for inclusion of 3 patients; a total of 3 patients should also still be included in sub-cohort 1c).
- **Sub-cohort 1d (half dose of EO4010), if opened, is planned to include 3 evaluable patients:**
 - If in 6 evaluable patients in total in sub-cohorts 1c + 1d, only 1 related Grade $\geq$ 3 AE (additional related Grade 3 AEs deemed not to constitute safety concerns by the IDMC are acceptable; see further above and below) occurred during the initial 4 weeks in the individual patients, the IDMC will assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (assumed would be opening recruitment of Cohort 2 if there are no general safety concerns; *note, that Cohorts 2, and 3, would include the lowered EO4010 dose*).
 - In case a related Grade 5 AE would occur in any of the patients recruited after the opening of sub-cohort 1d (*i.e.* any of the patients to fill sub-cohort 1c to a total of 3 patients or any of the 3 patients in sub-cohort 1d), recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation regarding further study conduct actions. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (recommendations might include terminating recruitment to the trial or depending on encountered safety events protocol adjustments to possibly allow addition of further safety cohorts; in addition, a recommendation regarding the further treatment, or not, of patients with ongoing trial medication would be foreseen).
 - If a related Grade 4 AE would occur in any of the patients recruited after the opening of sub-cohort 1d (*i.e.* any of the patients to fill sub-cohort 1c to a total of

3 patients or any of the 3 patients in sub-cohort 1d), EO4010 will be permanently stopped in the individual patient experiencing the event, and recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further recruitment steps (recommendations might include terminating recruitment to the trial or depending on encountered safety events protocol adjustments to possibly allow addition of further safety cohorts; in addition, a recommendation regarding the further treatment, or not, of patients with ongoing trial medication would be foreseen).

- If a related Grade 3 AE would occur in any of the patients recruited after the opening of sub-cohort 1c (*i.e.* any of the patients to fill sub-cohort 1c to a total of 3 patients or any of the 3 patients in sub-cohort 1d), EO4010 administration will be temporarily held in the individual patient experiencing the event, and recruitment to the trial will be held until a decision is made by the Sponsor following an IDMC recommendation. Thus, the IDMC will convene to assess the totality of available safety data and recommend for decision by the Sponsor the further treatment plan for the individual patient experiencing the event and further recruitment steps (the event might be deemed by the IDMC not to constitute a safety concern in which case the patient can continue treatment and recruitment continue per plan; alternatively, the IDMC might deem the event to constitute a safety concern in which case EO4010 will be permanently stopped in the patient experiencing the event and recommendations regarding the trial might include terminating recruitment, or depending on encountered safety events protocol adjustments to possibly allow addition of further safety cohorts; in addition, a recommendation regarding the further treatment, or not, of patients with ongoing trial medication would be foreseen).

4.2.4 Cohort 2 treatments

Cohort 2 (n = 15 evaluable patients) is planned to be initiated following a decision by the Sponsor after a recommendation from the IDMC based on the review of the safety and tolerability data from Cohort 1 (see above). The treatment in Cohort 2 should be EO4010 in combination with nivolumab from start of study treatment, and following the schedule of EO4010 in Cohort 1, *i.e.* [REDACTED] in the priming phase separated by [REDACTED], and after a [REDACTED] following administrations in the boosting phase on a [REDACTED] schedule.

4.2.5 Cohort 3 treatments

Cohort 3 (3a and 3b) (n = 10 evaluable patients in C3a and n=5 in C3b) will be initiated once the recruitment in the Cohort 2 is completed. After implementation of protocol amendment 1 (leading to EOCRC2-22 protocol version 2), Cohorts 3a and 3b, including patients with identical eligibility criteria plus criteria related to bevacizumab use, but having different treatment combination (EO4010/nivolumab/bevacizumab in Cohort 3a, EO4010 and bevacizumab in Cohort 3b), will be open for recruitment at the same time. Method of treatment assignment is described in [Section 6.7](#). The full dose (1 mL of EO4010 in adjuvant; 300 µg/peptide) of EO4010, defined based on the outcome of Cohort 1, should be used in Cohorts 2 and 3 and should be the same at every time of administration. The dose of nivolumab should be [REDACTED] for the first 3 administrations, and from the fourth administration, and onwards, a nivolumab dose of [REDACTED] is to be

applied. The dose of bevacizumab should be [REDACTED].

For all patients in Cohorts 2 and 3, the general safety rules as described above applies, *i.e.* at related clinical Grade 4/5 AEs the IDMC will convene and after assessing the situation give recommendations for further actions, and at related Grade 3 AEs the Investigator responsible for the patient with an event will in collaboration with the Sponsor make the initial assessment regarding actions; the IDMC support will be requested when appropriate.

4.3 End of study

4.3.1 Planned study completion and study duration

The clinical data cut-off for the final study analysis, is planned to be done at a time when the median survival, in Cohort 3 can be considered stable (*i.e.*, no longer possibly influenced by censored patients); the exact timing is not known, but based on data from the SUNLIGHT study a median follow-up of around [REDACTED] in favor of EO4010/nivolumab/bevacizumab might be needed (in SUNLIGHT the median follow-up was 14.2 months).

The assumed recruitment time is [REDACTED]. The draft 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 assumed to be [REDACTED] after the last patient enrolled; this time is an estimate and might change (no protocol amendment is assumed if within the expected margins of [REDACTED] after the last patient enrolled). The time from start of recruitment until last patient visit is assumed to be maximum [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.

4.3.2 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 EO4010.

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.



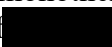
Reasons for the early closure of a study site by the Sponsor may include, but are not limited to:

- 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.

4.4 Discussion of study design

The trial is a multicenter, open-label, FIH, phase 1/2 trial to assess safety, tolerability, immunogenicity, and preliminary efficacy of the microbial-derived therapeutic vaccine EO4010 in combination with nivolumab, and/or with bevacizumab, for treatment of patients with unresectable, previously treated, metastatic CRC.

Previously treated metastatic CRC is not a rare disease, but at well-functioning sites with good trial conduct abilities a lot of development efforts are ongoing and thus to manage a relevantly short overall recruitment time it is necessary to utilize a multicenter approach even in a first-in-man trial as the current trial.

It is judged that the early development nature of the trial makes an open label design appropriate. Also, considering that the aim of the development of EO4010 is to define a treatment schedule including EO4010 and anti-PD-1 blockade based on learnings from the early development of EO2401 (see [Section 1.2.5](#)), it is judged that for the current trial in colorectal cancer, it is adequate to immediately target the combination, *i.e.* to not include any longer EO4010 monotherapy part in Cohort 1, except an acute safety assessing monotherapy period of .

For safety and tolerability, the main assessment items in the trial, the selected design is judged to have the possibility to stringently evaluate both early and late toxicity, of importance for an immunization approach, via the demand of thorough assessments of related Grade 3/4 AEs for all patients in all cohorts throughout all study treatments (see general safety rules in [Section 4.2](#)). Also, beside the active safety oversight of the trial by the Investigators (and their teams) in collaboration with the Sponsor via the Medical Monitor (with associated team), an IDMC will support the safety monitoring and give recommendations for possible actions in relation to their assessments of safety events.

The trial includes, due to the specific characteristics of the combination of EO4010 and nivolumab key well defined strategies for patient management at possible immune-mediated reactions (see [Section 6.5](#) and extended treatment algorithms in [Section 12.6](#)).

It is acknowledged that the trial is intensive regarding sampling. However, a key parameter for evaluation in the trial is to analyze EO4010-induced immunity and the tests for such analyzes unfortunately demand relatively high volumes of blood for adequate tests to be run (*e.g.* PBMC); a minimization strategy with regard to sampling has been applied, still without risking the appropriate possibility to get relevant readouts. The future development of EO4010 will be directed by the outcome of the analyses of induced immunity in the early development trials.

Preliminary efficacy is also to be assessed in the trial and as indicated RECIST 1.1 ([Eisenhauer et al., 2009](#)) and iRECIST ([Seymour et al., 2017](#)) criteria will be used for response assessments (local evaluations of response parameters are going to be utilized considering the early development nature of the trial). The basis for this decision is that the novel mechanism of action of the compounds used in the current trial, EO4010 in



combination with PD-1 blockade, *i.e.* a treatment combination aiming at immune and T cell activation, is postulated to lead to unusual patterns of response that might resemble tumor flare ([Eisenhauer et al., 2009](#)). In early trials of immune-based therapeutics in melanoma, investigators described unique response patterns, termed pseudoprogession. Some patients whose disease met the criteria for disease progression based on traditional response criteria such as RECIST (an increase in the sum of measures of target lesions, unequivocal increase in non-target disease, or the appearance of new lesions) were noted to have late but deep and durable responses ([Eisenhauer et al., 2009](#)). Therefore, response and progression in this trial will be evaluated using the revised international criteria (1.1) proposed by the RECIST (Response Evaluation Criteria in Solid Tumors) committee ([Eisenhauer et al., 2009](#)) as well as the modified iRECIST guidelines ([Seymour et al., 2017](#)).



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 Protocol [Section 7.2](#) and [Section 7.3](#), respectively). Patients must be able to understand and be willing to sign a written informed consent.
2. Histological confirmation of advanced non-resectable colorectal adenocarcinoma (confirmation at initial diagnosis is sufficient) which is mismatch repair proficient and microsatellite stable (according to local site standard testing procedures), *i.e.* patients with mismatch repair deficient or microsatellite instability-high metastatic disease cannot be recruited to this trial.
3. Patients with metastatic colorectal cancer who have been previously treated with, or are not considered candidates for (according to the judgement of the recruiting site physician; *e.g.*, due to progression or unacceptable toxicity on the type of treatment, or type of treatment not indicated in the specific patient due to his/her disease characteristics - when appropriate a collaborative discussion between the recruiting physician and the Medical Monitor should be held to keep the study population consistent), therapies including fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapies, anti-VEGF agents, and anti-EGFR agents.
4. Progression during or within 3 months following the latest administration of standard therapies as outlined in inclusion criterion #3.
5. Age $\geq$ 18 years old.
6. Human leukocyte antigen (HLA)-A2 positive.
7. ECOG performance status 0 or 1 (see Protocol [Section 12.1](#)).
8. Measurable disease according to Response Evaluation Criteria in Solid Tumors criteria (RECIST), version 1.1.
9. Patients with a life expectancy of at least 3 months as judged by the recruiting site physician.
10. Female patients of childbearing potential must have a negative serum pregnancy test within 72 hours prior to first administration of study drug.
11. 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 ([Heads of Medicines Agencies: Clinical Trials Facilitation and Coordination Group, n.d.](#); [Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)):
 - 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. *Note, the contraception guidelines for males are not related to nivolumab but kept as a conservative precaution in this early development protocol in relation to EO4010.*
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 first administration of study drug, unless required to treat an adverse event (AE).

2. Patients treated with radiotherapy within 12 weeks, and cytotoxic chemotherapy therapy within 28 days (or 5 halflives 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 who have previously received trifluridine/tipiracil (TAS-102) or regorafenib.
6. Patients with prior exposure to EO2401, EO2040, or EO4010, *i.e.* therapeutic vaccine compounds including all or some components of EO4010, the current trial compound.
7. Patients with the following abnormal laboratory values:

a.

8. Other malignancy or prior malignancy with a disease-free interval of less than 3 years prior to ICF signing: [REDACTED]

9. Patients with clinically significant [REDACTED], cardiac disease [REDACTED] 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. [REDACTED]

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

- [REDACTED]
- [REDACTED]
12. Patients with a history of solid organ transplantation or allogeneic hematopoietic stem cell transplantation.
13. [REDACTED].
14. Pregnant and breastfeeding patients.
15. Patients with a history or [REDACTED]
16. Uncontrolled central nervous system (CNS) metastasis; [REDACTED]
17. [REDACTED]
18. Patients with a history of hypersensitivity to any excipient, or active substance, present in the pharmaceutical forms of applicable study treatments.
19. [REDACTED]
20. 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 first administration of study drug. 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).
21. [REDACTED]



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.

In instances where patient consent is withdrawn, the Investigator must clarify to which degree the patient consent is withdrawn, i.e. whether the patient still is willing to continue to participate in some study specific procedures, or not. The degree of consent withdrawal should be carefully documented in patient files and in study documents.

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. For documentation reasons all study related items performed before the determination of the status of screen failure should be documented in the eCRF.

5.3.3 Criteria for treatment discontinuation

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

- Reached individual maximum defined study treatment period (EO4010 in combination with nivolumab and/or bevacizumab) of 24 months,
- intolerable toxicity in relation to study treatment and adverse events requiring treatment discontinuation according to this protocol (see [Section 4.2](#) 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 4.3](#)).

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 4.3](#)).

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](#). The first follow-up visit is to be completed 30 days after completion of last dose of study treatment.

In addition to the 30-days visit, for consistency with other nivolumab clinical trial protocols as advised by Bristol-Myers Squibb (marketing authorization holder for nivolumab),  visit following discontinuation of nivolumab treatment is also targeted.

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. However, the primary analysis for each cohort builds on an assessment of 15 patients (10 patients in 3a and 5 patients in 3b arms) in the Full Analysis Set (FAS; patients who received at least one dose of EO4010 and for whom no important protocol deviations occurred). Thus, if there would be relevant important protocol deviation(s) in a patient impacting for instance the possibility to assess the primary endpoint the patient might be replaced based on a decision by the Sponsor.

In addition, in Cohort 1, if for reasons other than safety a patient does not receive 2 doses of EO4010 and complete the [REDACTED] the patient will not be counted towards [REDACTED]. Patients who are not evaluable for [REDACTED] in Cohort 1 will be replaced for [REDACTED] assessment; however, if such a patient has received at least one dose of EO4010 he/she should be evaluated in the Safety Population.



6 TREATMENT OF PATIENTS

6.1 Description of EO4010

6.1.1 *Pharmaceutical form*

The Investigational Medicinal Product (IMP) of the study, EO4010 Drug Product (DP), consists of a solution of 6 peptide drug substances (EO2317, EO2318, OMP10, OMP11, OMP12, and UCP2) in a WFI/DMSO solvent mixture. The EO4010 DP is then extemporaneously [REDACTED] with the [REDACTED] to form a water-in-oil emulsion for subcutaneous injection. The final concentration of each peptide in the emulsified DP in adjuvant is 300 µg/mL.

Further details regarding the components of EO4010 can be found in [Section 1.2](#), and in the current version of the EO4010 IB.

6.1.2 *Preparation of the emulsion*

The IMP is provided to the clinical sites in a form of a mixing kit containing 2 vials (1 EO4010 DP vial and [REDACTED]) plus additional reconstitution material. The Investigator or pharmacist at the investigational site will undertake specific training on handling and preparing study treatment and will ensure that Good Pharmacy Practices are followed during the preparation and reconstitution process of EO4010.

Detailed composition of EO4010 is presented in [Table 2](#).

Detailed instructions for the reconstitution of emulsified EO4010 DP [REDACTED] [REDACTED] will be provided in the Pharmacy Manual.

Table 2: Composition of the final emulsified EO4010 DP in adjuvant obtained by reconstitution

No.	Code	Component vial	Function in formulation	Amount	
				Total*	Per 1 mL (SC single dose)
1	EO2317	EO4010 DP vial	Drug substance	0.36 mg	0.30 mg
2	EO2318		Drug substance	0.36 mg	0.30 mg
3	OMP10		Drug substance	0.36 mg	0.30 mg
4	OMP11		Drug substance	0.36 mg	0.30 mg
5	OMP12		Drug substance	0.36 mg	0.30 mg
6	UCP2		Drug substance	0.36 mg	0.30 mg

*Referring to the volume of 600 µL used for reconstitution.

- **EO4010 vial:** (a sterile solution in a glass vial) comprises EO4010 DP, the 6-peptide mixture solution, each at 600 µg/mL [REDACTED], intended for proprietary use in this clinical trial with EO4010. It contains an extractable volume of not less than 0.6 mL solution for the reconstitution process to obtain the final IMP EO4010 DP [REDACTED].
- [REDACTED]
- Regarding additional material see [Section 6.1.3](#).

Reconstitution process

The final emulsified EO4010 DP in adjuvant will be reconstituted by the investigational site pharmacy, following the Pharmacy Manual and the related instructions for reconstitution for the [REDACTED] EO4010 DP [REDACTED].

The final emulsified EO4010 DP [REDACTED] will be obtained by [REDACTED] 600 µL of the EO4010 DP, the peptide mixture solution, [REDACTED] solution using a 2-syringe mixing process. In the starting sub-cohort 1a, up to 1 mL of the resulting emulsion of EO4010 will be administered as an SC injection within 4 hours from the start of the reconstitution (*i.e.* from the start of mixing EO4010 and [REDACTED]). This administration volume will contain a dose of 300 µg of each of the 6 peptides, corresponding to a total peptide drug substance dose of 1.8 mg, accordingly. The composition of the final [REDACTED] EO4010 DP in adjuvant obtained by reconstitution is provided in [Table 2](#). Training on the reconstitution process will be given.

The final emulsion must be cautiously delivered, *e.g.* do not deliver/transport the final emulsion via pneumatic tube systems, to avoid splitting the drug emulsion.

6.1.3 Packaging and labelling

EO4010 will be manufactured as per Good Manufacturing Practice (GMP) and supplied by the Sponsor as a mixing kit composed of 2 boxes:

- 1 box containing EO4010 DP vial which is a sterile 2R Type I glass vial containing 0.85 mL of peptide mix solution,

- [REDACTED]

EO4010 DP vial and secondary packaging will be labeled with a study-specific primary label. Labels with requirements for the specific country will also be applied. The [REDACTED] vial [REDACTED]) will be labeled with the original primary label of commercial supplier. The secondary packaging (*i.e.* carton box) will be labeled with a study-specific secondary label, which will state storage conditions. Label text will be approved by the Sponsor and include specific country requirements.

For all vials, a system in accordance with GMP will be used; ensuring each dose of EO4010 can be traced back to the respective bulk ware of the ingredients. 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. The study treatment is presented as 2-boxes mixing kit that must be stored as per the following requirements:

- Box with EO4010 DP vials to be stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$.

- [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.

Nivolumab concentrate is presented in a Type I glass vial with a coated butyl rubber stopper and an aluminum flip-off seal.

List of excipients includes 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 ([Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)).

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 ([Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)).

6.2.3 Packaging and labeling

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

6.2.4 Adequate supplies of nivolumab glass vials will be provided to each investigational site for preparation and infusion. Storage and stability

Nivolumab should be stored in the refrigerator at $5 \pm 3^{\circ}\text{C}$ (do not freeze) in its original package in order to protect from light. The unopened vial can be stored at controlled room temperature up to 25°C with room light for up to 48 hours. Once opened, the medicinal product should be infused or diluted, and infused immediately ([Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)).

If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 7 days at 2°C to 8°C or 8 hours (of the total 7 days of storage) at room temperature ($\leq 25^{\circ}\text{C}$). Aseptic handling should be ensured during the preparation of infusion.

6.2.5 Expected safety profile of nivolumab

The following summary is based on the European SmPC ([Opdivo | European Medicines Agency, 2023](#)).

In the pooled dataset of nivolumab as monotherapy across tumour types ($n = 4646$) with minimum follow-up ranging from 2.3 to 28 months, the most frequent adverse reactions ($\geq 10\%$) were fatigue (44%), musculoskeletal pain (28%), diarrhoea (26%), rash (24%), cough (22%), nausea (22%), pruritus (19%), decreased appetite (17%), arthralgia (17%), constipation (16%), dyspnoea (16%), abdominal pain (15%), upper respiratory tract infection (15%), pyrexia (13%), headache (13%), anaemia (13%) and vomiting (12%). The majority of adverse reactions were mild to moderate (Grade 1 or 2). The incidence of Grade 3-5 adverse reactions was 44%, with 0.3% fatal adverse reactions attributed to study drug. 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 3** 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 ([Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)).

Of the 3529 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, 328 patients (9.3%)



tested positive for treatment-emergent anti-product-antibodies with 21 patients (0.6%) testing positive for neutralizing antibodies ([Opdivo | European Medicines Agency, 2023](#)).

Table 3: 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 reactions leading to permanent discontinuation	
Pneumonitis	1.4
Colitis	12
Hepatitis	1.1
Nephritis and renal dysfunction	0.3
Endocrinopathies	0.5
Skin	0.8
Hypersensitivity/Infusion reaction	0.1
Immune-related adverse reactions requiring high-dose corticosteroids ^{a,b}	
Pneumonitis	65
Colitis	14
Hepatitis	21
Nephritis and renal dysfunction	22
Endocrinopathies	5
Skin	3.3
Hypersensitivity/Infusion reaction	18
^a at least 40 mg daily prednisone equivalents; ^b frequency is based on the number of patients who experienced the immune-related adverse reaction	

6.3 Description of bevacizumab

6.3.1 Pharmaceutical form

Bevacizumab is a recombinant humanised monoclonal antibody produced by DNA technology in Chinese Hamster Ovary cells. Presented as a concentrate for solution for infusion. The solution is clear to slightly opalescent, colourless to pale brown liquid. Bevacizumab concentrate is presented in a Type I glass vial with a butyl rubber stopper.

Further details regarding bevacizumab can be found in the European SmPC or the US PI ([Zirabev | European Medicines Agency, 2023](#); [Zirabev-Drugs@FDA: FDA-Approved Drugs February 2023, n.d.](#));

6.3.2 Preparation of the formulation

Instructions for preparation of bevacizumab for infusion can be found in the European SmPC or the US PI for bevacizumab ([Zirabev | European Medicines Agency, 2023](#); [Zirabev-Drugs@FDA: FDA-Approved Drugs February 2023, n.d.](#)).



No incompatibilities between bevacizumab and polyvinylchloride or polyolefin bags have been observed.

Bevacizumab at 5 mg/kg every 2 weeks will only be administered to patients recruited to Cohort 3 (3a and 3b).

6.3.3 Packaging and labeling

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

Adequate supplies of bevacizumab will be provided to each investigational site.

6.3.4 Storage and stability

Bevacizumab must be stored in the refrigerator at $5 \pm 3^{\circ}\text{C}$ (do not freeze) in its original package in order to protect from light. Once opened, the medicinal product should be infused or diluted, and infused immediately ([Zirabev | European Medicines Agency, 2023; Zirabev-Drugs@FDA: FDA-Approved Drugs February 2023, n.d.](#)). The concentration after dilution in 0.9% sodium chloride must be kept with the range of 1.4 mg/ml and 16.5 mg/ml.

After dilution, chemical and physical in-use stability has been demonstrated for a period of up to 35 days at 2°C to 8°C and a period of up to 48 hours at temperatures not exceeding 30°C in sodium chloride 9 mg/ml (0.9%) solution for injection. From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions are the responsibility of the user and would normally not be longer than 24 hours at 2°C to 8°C , unless dilution has taken place in controlled and validated aseptic conditions.

6.3.5 Expected safety profile of bevacizumab

The following summary is based on the EU SmPC of Zirabev.

The overall safety profile of bevacizumab is based on data from over 5700 patients with various malignancies, predominantly treated with bevacizumab in combination with chemotherapy in clinical trials.

The most SAEs were:

- Gastrointestinal perforations
- Hemorrhage, including pulmonary hemorrhage/hemoptysis, which is more common in non-small cell lung cancer patients
- Arterial thromboembolism

The most frequently observed adverse reactions across clinical trials in patients receiving bevacizumab were hypertension, fatigue or asthenia, diarrhea, and abdominal pain. Analyses of the clinical safety data suggest that the occurrence of hypertension and proteinuria with bevacizumab therapy are likely to be dose-dependent.

6.4 Administration of EO4010 and the combination of EO4010 with nivolumab and/or with bevacizumab

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

- EO4010 should be injected within 4 hours from the start of the reconstitution (*i.e.* from the start of mixing EO4010 peptide mix solution and [REDACTED])
 - EO4010 (up to 1 mL of the emulsion) should be administered subcutaneously (SC) in a rotating way by injection (thus, an injection site will be used every fourth time), with the [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]!
- EO4010 will during the initial [REDACTED] be administered SC 3 times, each administration followed by [REDACTED] observation period. After the initial 6 weeks EO4010 will be administered with a [REDACTED] 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 EO4010 administration.
 - Dose modifications for EO4010 include withholding of doses and treatment discontinuation (see [Section 6.4](#)).
 - At combination treatment when EO4010 and nivolumab are to be administered on the same day, the order of administration is to start with EO4010, followed by a [REDACTED] observation period, after which the nivolumab infusion should be administered intravenously (IV) according to local guidelines. The dose of nivolumab should be 240 mg at [REDACTED] and [REDACTED] and from [REDACTED] and onwards 480 mg per administration [REDACTED]. Observation after nivolumab administration should follow local guidelines.
 - Cohort 3 (3a and 3b) is planned to be initiated after recruitment in the Cohort 2 is completed. It will include administration of EO4010 in combination with nivolumab and bevacizumab in 3a arm and administration of EO4010 in combination with bevacizumab in 3b arm from the start of treatment in the individual patients. EO4010 and nivolumab administration schedules and doses are the same as in Cohort 2.
 - Bevacizumab will be administered as an IV infusion at a dose of [REDACTED] after the start of nivolumab administration per local practice in 3a arm and 2 hours after an EO4010 administration in 3b arm. 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 (*Opdivo | European Medicines Agency, 2023; Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.*); see also [Section 6.4](#).



- The dose modifications for bevacizumab should be taken in accordance with European SmPC or the US PI ([Zirabev | European Medicines Agency, 2023](#); [Zirabev-Drugs@FDA: FDA-Approved Drugs February 2023, n.d.](#))
- In case of infusion-related reactions and hypersensitivity reactions, please refer to [Section 12.4](#).
- In case of immune-related adverse events and local administration site reactions related to study treatments, instructions for treatment modifications as outlined in [Section 6.5](#) should be followed.

6.5 Treatment modifications for EO4010, nivolumab and bevacizumab

- At related Grade ≥ 3 adverse events special procedures are to be followed, see [Section 4.2](#).
- Dose modifications for EO4010 include withholding of doses and treatment discontinuation (see [Section 4.2](#), [Section 5.3.3](#), [Section 6.5.1](#) and [Section 6.5.2](#)).
- 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 ([Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)); see also [Section 4.2](#), [Section 5.3.3](#), [Section 6.5.1](#), [Section 6.5.2](#), and [Section 12.6](#).
- In instances where the label(s) for nivolumab prescribe that the drug should be withheld or discontinued due to safety events, EO4010 might be continued, provided the treating physician considers this to be safe for the patient, and the continuation is approved by the Sponsor's Medical Monitor.

Dose adjustments are necessary to consider in the case of administration-related reactions (see [Section 12.4](#)).

In case of immune-related adverse events related to EO4010 and/or nivolumab, instructions for treatment modifications as outlined in [Section 6.5.1](#) should be followed.

In case of local administration site reactions related to EO4010 and/or nivolumab and/or bevacizumab, instructions for treatment modifications as outlined in [Section 6.5.3](#) should be followed.

General criteria for treatment discontinuation and study participation termination are outlined in [Section 5.3](#).

6.5.1 Recommended treatment modifications for EO4010 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 4](#) 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 (*Opdivo | European Medicines Agency, 2023; Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.*).

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. There is no such timeline established for EO4010. However, it is assumed that the monitoring period for nivolumab would also be adequate for EO4010.

Patients will be reviewed on a case-by-case basis, should they need to discontinue EO4010 and/or nivolumab due to toxicity. See [Section 4.2](#) and [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 ([Haanen et al., 2017](#))
- 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 ([Schneider et al., 2021](#))
- 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. (*NCCN Clinical Practice Guidelines in Oncology: Management of Immunotherapy-Related Toxicities. Version 4.2021 - September 27, 2021, n.d.*)

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 ([Haanen et al., 2017; NCCN Clinical Practice Guidelines in Oncology: Management of Immunotherapy-Related Toxicities. Version 4.2021 - September 27, 2021, n.d.; Schneider et al., 2021](#)). In addition, the European SmPC and the US PI also includes treatment recommendations for some immune-related immune reactions (*Opdivo | European Medicines Agency, 2023; Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.*), and [Section 12.6](#) outlines management algorithms regarding nivolumab for some specific adverse events.



Table 4: Recommended treatment modifications for EO4010 and nivolumab in relation to immune-related adverse reactions

Immune-related adverse reaction	NCI-CTCAE v. 5.0 (123)	Treatment action
Immune-related pneumonitis	Grade 2 pneumonitis	Withhold dose(s) until symptoms resolve, radiographic abnormalities improve, and management with corticosteroids or empirical antibiotics is complete.
	Grades 3 and 4 pneumonitis	Permanently discontinue treatment.
Immune-related colitis	Grade 2 diarrhea or colitis	Withhold dose(s) until symptoms resolve and management with corticosteroids, if needed, is complete.
	Grade 3 diarrhea or colitis	Withhold dose(s) until symptoms resolve and management with corticosteroids is complete.
	Grade 4 diarrhea or colitis	Permanently discontinue treatment.
Immune-related hepatitis	AST or ALT > 3.0 to $\leq 5 \times$ ULN, total bilirubin > 1.5 to $\leq 3.0 \times$ ULN	Withhold dose(s) until laboratory values return to baseline and management with corticosteroids, if needed, is complete.
	AST or ALT $> 5 \times$ ULN. Total bilirubin $> 3 \times$ ULN	Permanently discontinue treatment.
Immune-related nephritis and renal dysfunction	Grade 2 ($>1.5 - 3.0 \times$ baseline; $>1.5 - 3.0 \times$ ULN) or Grade 3 ($>3.0 \times$ baseline; $>3.0 - 6.0 \times$ ULN) creatinine elevation	Withhold dose(s) until creatinine returns to baseline and management with corticosteroids is complete.
	Grade 4 ($>6.0 \times$ ULN) creatinine elevation	Permanently discontinue treatment.
Immune-related endocrinopathies	Symptomatic Grade 2 or 3 hypothyroidism, hyperthyroidism	Withhold dose(s) until symptoms resolve and management with corticosteroids (if needed for symptoms of acute inflammation) is complete. Treatment should be continued in the presence of hormone replacement therapy as long as no symptoms are present.
	* Grade 1-3 hypophysitis/hypopituitarism that is also associated with corresponding abnormal lab and/or pituitary scan Grade 2 adrenal insufficiency	



Immune-related adverse reaction	NCI-CTCAE v. 5.0 (123)	Treatment action
	Grade 3 diabetes * Grade 2 or 3 requiring initiation or change in daily management	
	Grade 4 hypothyroidism Grade 4 hyperthyroidism * Grade 4 hypophysitis/hypopituitarism * Grade 3 or 4 adrenal insufficiency or adrenal crisis Grade 4 diabetes	Permanently discontinue treatment.
Immune-related skin adverse reactions	* Grade 2 rash covering > 30% body surface area	Withhold dose(s) until rash reduces to ≤ 10% body surface area
	Grade 3 rash	Withhold dose(s) until symptoms resolve and management with corticosteroids is complete.
	Grade 4 rash	Permanently discontinue treatment.
	Stevens-Johnson syndrome or toxic epidermal necrolysis * or drug reaction with eosinophilia and systemic symptoms (DRESS)	Permanently discontinue treatment.
* Immune-mediated encephalitis	Monitor for changes in neurologic function	Withhold for new-onset moderate to severe neurological signs or symptoms provided the symptoms are not related to the patients underlying CNS malignancy, or study treatment mechanism based immune attack on the tumor under treatment (in the latter cases standard of care for CNS malignancies should be implemented. Permanently discontinue study treatment for any grade drug related encephalitis.
* Guillain-Barre Syndrome	Any grade	Permanently discontinue treatment.
* Myasthenia Gravis	Any grade	Permanently discontinue treatment.
	Myelitis, any grade	Withhold dose(s), after workup if myelitis is not drug related,



Immune-related adverse reaction	NCI-CTCAE v. 5.0 (123)	Treatment action
* Other potentially immune-mediated neurological diseases		treatment may resume when AE resolves.
	Myelitis, any grade drug-related	Permanently discontinue treatment.
	Other condition, grade 2	Withhold dose(s), treatment may resume when AE resolves to baseline.
	Other condition, grade 3 or 4	Permanently discontinue treatment.
* Immune-related uveitis	Grade 2 uveitis	Withhold dose(s), treatment may resume if uveitis respond to topical therapy (eye drops) and uveitis resolves to Grade ≤ 1 or baseline. If patient requires oral steroids for uveitis, then permanently discontinue treatment.
	Grade 3 or 4 uveitis	Permanently discontinue treatment.
Immune-related myocarditis	Grade 2 myocarditis; * symptoms induced from mild to moderate activity exertion	Withhold dose(s) until symptoms resolve and management with corticosteroids is complete.
	Grade 3 or 4 myocarditis; * severe or life threatening, with symptoms at rest or with minimal activity or exertion, and/or where intervention indicated	Permanently discontinue treatment
Other immune-related adverse reactions <i>NOTE, for Local Administration Site Reactions follow Section 6.5.3</i>	Grade 3 (first occurrence)	Withhold dose(s).
	Grade 4 or recurrent Grade 3; persistent Grade 2 or 3 despite treatment modification; inability to reduce corticosteroid dose to 10 mg prednisone or equivalent per day	Permanently discontinue treatment.

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase, NCI-CTCAE = National Cancer Institute-Common Terminology Criteria for Adverse Events; ULN = upper limit of normal.



Source: Opdivo SmPC ([Opdivo | European Medicines Agency, 2023](#)), also stating toxicity grades are in accordance with NCI-CTCAE v5.0, and when text preceded by * updated based on standard protocol guidance from Bristol-Myers Squibb (market authorization holder for Opdivo).

6.5.2 Recommended treatment modifications for Adverse Reactions to bevacizumab

Details of dose modifications for adverse reactions to bevacizumab are provided in [Table 5](#).

Table 5: Dose Modifications for Adverse Reactions to Bevacizumab

Adverse Reaction	Severity	Dose Modification
Gastrointestinal Perforation and fistulae	- Gastrointestinal perforation, any grade - Tracheoesophageal fistulae, any grade - Fistulae, Grade 4 - Fistulae formation involving any internal organ	Discontinue bevacizumab
Wound Healing Complications	- Wound healing complications requiring medical intervention - Necrotizing fasciitis	Discontinue bevacizumab
Hemorrhage	Grade 3 or 4	Discontinue bevacizumab
	Recent history of hemoptysis of ½ teaspoon (2.5 mL) or more	Withhold bevacizumab
Thromboembolic Events	Arterial thromboembolism, severe	Discontinue bevacizumab
	Venous thromboembolism, Grade 4	Discontinue bevacizumab
Hypertension	- Hypertensive crisis - Hypertensive encephalopathy	Discontinue bevacizumab
	Hypertension, severe	Withhold bevacizumab if not controlled with medical management; resume once controlled
Posterior Reversible Encephalopathy Syndrome	Any	Discontinue bevacizumab
Renal Toxicity and Proteinuria	Nephrotic syndrome	Discontinue bevacizumab
	Proteinuria greater than or equal to 2 g per 24 hours in absence of nephrotic syndrome	Withhold bevacizumab until proteinuria less than 2 g per 24 hours
Infusion Reaction	Severe infusions reaction	Discontinue bevacizumab
	Clinically significant	Interrupt infusion; resume at a decreased rate of

Adverse Reaction	Severity	Dose Modification
		infusion after symptoms resolve
	• Mild, clinically insignificant	Decrease infusion rate
Congestive Heart Failure	• Any	Discontinue bevacizumab

Reference source: ([Zirabev-Drugs@FDA: FDA-Approved Drugs February 2023, n.d.](#))

6.5.3 Recommended treatment modifications for EO4010, nivolumab and bevacizumab in case of local administration site reactions

Local administration site reaction (LASR) is the most distinct event type linked to the microbiome-derived therapeutic vaccines which are currently in clinic trials, and the only AE type which is more common for the combination of EO2401 () plus nivolumab versus using nivolumab monotherapy.

The recommendations given below builds on the experience gained in clinical trials utilizing EO2401 and are to be applied in all clinical trials including Enterome microbiome-derived therapeutic vaccines until further information is obtained from clinical experience if such information would lead to added mitigation.

The recommended NCI CTCAE terms (v5.0) to use for grading of AEs of LASR are “Injection site reaction” or “Vaccination complication” ([Table 6](#)).

Table 6: Recommended CTCAE terms for use for Local Administration Site Reactions
[[Common Terminology Criteria for Adverse Events \(CTCAE\) v5.0 Quick Reference 8.5x11, 2017](#)]

General disorders and administration site conditions					
CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Injection site reaction	Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death
Definition: A disorder characterized by an intense adverse reaction (usually immunologic) developing at the site of an injection.					
Navigational Note: -					
Vaccination complication	Mild pain; erythema 2.5-5cm; induration/swelling 2.5-5cm; does not interfere with activity	Moderate pain; Erythema 5.1-10 cm; Induration/swelling 5.1-10 cm; lipodystrophy; limiting instrumental ADL	Severe pain; Erythema > 10 cm; Induration/swelling > 10 cm; necrosis; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	-
Definition: A disorder that occurs after the injection of a substance with antigenic properties, administered to activate the immune system.					
Navigational Note: For systemic vaccination complications, consider immune system disorders: Allergic reaction or Anaphylaxis.					

LASR is associated with the use of () and is an expected event type in clinical trials with the microbiome-derived compound EO2401. The frequency of LASRs seen on the EOGBM1-18 and EOADR1-19 trials are below, or within the expected frequency for any antigen administered together () as an adjuvant.

Of note, LASRs in the context of the compound EO2401 seem linked to the strength of the immune response achieved, and treatment duration, meaning that preliminary analyses suggest that patients with better therapy outcomes are more prone to experience LASRs. However, there are also patients treated with EO2401 without any sign of LASR who

experience tumor shrinkage at study treatment, and there are patients with LASR who do not experience any anti-tumor effect.

Summary regarding Local Administration Site Reactions (LASR) in trials utilizing the microbiome-derived compound EO2401 and [REDACTED] in combination with nivolumab and bevacizumab EOGBM1-18 and EOADR1-19 is provided in [Section 1.2.5.1 EO2401 clinical safety experience](#).

To document a LASR it is strongly recommended, that in addition to the reporting of the AE, the lesion(s) are also **described at each visit in the eCRF physical examination pages**.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Overall, by experience from the compound EO2401 the absolute majority of events are Grade 1 and Grade 2 which usually do not need any supportive treatment at all. A typical appearance and disappearance at one location of a Grade 1/2 LASR is given in [Figure 19](#), and this event did not need any treatment. However, these Grade 1/2 events may also give rise to intermittent pain and if needed the recommended firsthand treatment option is then paracetamol/acetaminophen.

The types of reactions as exemplified in [Figure 19](#) are typically sterile and thus do not necessitate topical or systemic antibiotic treatment, however there may be clinical situations where treatment with antibiotics is indicated and this should be determined by the treating clinician; if in doubt, the Medical Monitor for the Sponsor can be contacted for discussion of such intervention.

NOTE, for Grade 1 or 2 lesions as in [Figure 19](#), there is usually [REDACTED]

[REDACTED] s. [REDACTED]. If there is a question about possible diagnostic or invasive interventions, please contact the Medical Monitor of the Sponsor for a discussion.

[REDACTED]

[REDACTED]

[REDACTED], among more than 165 patients treated with the compound EO2401, there are eleven patients with reported study treatment interruptions due to LASRs including nine with Grade 3 documented ulcerations at one or multiple injection sites. The sometimes fast (after an administration of the therapeutic vaccine EO2401) development of an ulceration is exemplified in [REDACTED]. In such cases, previously injected sites can worsen even if the most recent administration is at a separate site. This likely reflect an immune-mediated attack where persistent peptides from the prior vaccine site may still reside within an old administration site. [REDACTED]

If an ulceration (Grade 3) occurs, all study treatments, i.e., both the therapeutic vaccine and anti-PD1 blockade should be immediately suspended until the lesion(s) heal. Further study therapy should be interrupted for a minimum [REDACTED] and until any ulcer has fully healed. After this time point, study therapy may resume if erythema or induration persist as long as the ulcer has resolved. [REDACTED] means that at last one administration time point for the vaccine [REDACTED] (combination treatments should be withheld during the same time period) if in the boosting period of the study (which is the assumed time for appearance of Grade 3 events). [REDACTED], contact the Medical Monitor for a discussion regarding the appropriate interruption period.

[REDACTED]

[REDACTED]

Of note, at this point there is NO predictive value of any feature which will predict which Grade 2 lesions will progress to Grade 3 ulcerations at local administration sites. For instance, the lesions at day -6 in [REDACTED] are not predictive for becoming ulcers and they may simply progress to subcutaneous indurations. **The lack of predictive factors, especially clinical, makes it of utmost importance to make all efforts to document LASRs as accurately as possible per the guidance given above so that future guidance may potentially be refined!**

[REDACTED]

Summary of guidance regarding Local Administration Site Reactions (LASR)

- To minimize the risk of developing an LASR, follow study protocol vaccine administration instructions including that:

- The vaccine () should be administered subcutaneously in

- If there is a local administration site reaction, document as thoroughly as possible via eCRF pages for adverse event and physical exam (see above).
- If Grade 1/2 LASR, continue the study vaccine administration using the described rotating schedule and . For any questions on how to handle these events, please contact the Medical Monitor at the Sponsor.

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- **For any Grade 3 LASR, ALL STUDY TREATMENTS (i.e. the therapeutic vaccine and all combination treatments, including anti-PD1 blockade) should be . Study therapy may be resumed if erythema or induration persist once the ulcer has healed. In such cases, all study therapy should be interrupted for a meaning that at least one administration time point for the vaccine/anti-PD1-blockade will be withheld if in the boosting period of the study** (which is the assumed time for appearance of Grade 3 events). If a Grade 3 event occurs during the of study treatment, please contact the Medical Monitor for a discussion regarding the appropriate interruption period.

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- Following interruption of study therapy due to a Grade 3 LASR, reactions to study therapy resumption can guide the frequency of subsequent vaccine doses.
 - If after the re-administration of study treatment there is recurrence of a Grade 1 or 2 LASR, the next, and continued administrations can be planned with a  interval between doses.
 - If after re-administration there is a new Grade 3 event, the patient needs a longer individualized time of study treatment interruption, and the patient should be discussed with the Medical Monitor for the Sponsor.
 - If a Grade 4 LASR occurs, further study treatment must be permanently discontinued.

6.6 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.



6.7 Method of treatment assignment

This is an open label (*i.e.* no blinding procedure will be implemented), non-randomized trial and each at the site newly enrolled patient (upon signing the ICF) will be assigned a unique patient identification number (identifying trial, site, and patient). After screening at the site regarding eligibility and final assessment by the site PI (or designee) documented in an approval of start of study treatment form which will be send the Sponsor Medical Monitor, there will be a Sponsor approval to start study treatment according to a separate Enrollment Management Plan.

After implementation of protocol amendment n°1 (leading to EOCRC2-22 protocol version 2) Cohorts 3a and 3b, including patients with identical eligibility criteria but having different treatment combination (EO4010/nivolumab/bevacizumab in Cohort 3a, EO4010 and bevacizumab in Cohort 3b), will be opened for recruitment at the same time. To manage a structured recruitment the Sponsor Medical Monitor will at request for screening slot from trial sites assign every other slot to Cohort 3a and Cohort 3b, respectively, until there are no more available slots for one of the cohorts, at which time all screening slots will be assigned to the cohort with available slots. Once a patient has been assigned a screening slot for one of the cohorts, the cohort assignment will be kept by the individual patient; in case the patient would screen-fail, for instance due to HLA-A2 negative results, the screening slot in the cohort will be open for a new patient.

Of special note:

- If screening laboratory procedures were done within 72 hours prior to Visit 1, they do not need to be repeated at V1 unless it should be done per the Investigator's opinion. If not done within 72 hours before V1, they need to be repeated at V1, and ***if there is a change of laboratory values from screening until V1 making the patient non-eligible the treating physician MUST not initiate treatment and inform the Enterome Medical Monitor immediately for decision on further actions.***

6.8 Ancillary treatments

6.8.1 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.

6.8.2 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 6.8.3](#). 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 6.5](#) and [Section 12.6](#)). 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 (EO4010 administered every 4 weeks), 2 weeks after the latest EO4010 administration (and thereby 2 weeks before the next EO4010 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 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), if further vaccines are approved for use at the time a study participant needs to be vaccinated the below table can be used for guidance regarding acceptable vaccines (if in doubt consult with the Medical Monitor of the Sponsor):

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.

6.8.3 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 6.8.2](#).





During trial participation patients should not receive any other anti-CRC treatment than the specified trial treatments, and treatments outlined in [Section 6.8.2](#).

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.

6.8.4 Contraception

For the purpose of this protocol, a woman is considered of childbearing potential, i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy.

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

For the purpose of this protocol, a man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

Potential postmenopausal status for female patients, per above definition, 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](#).

6.9 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.

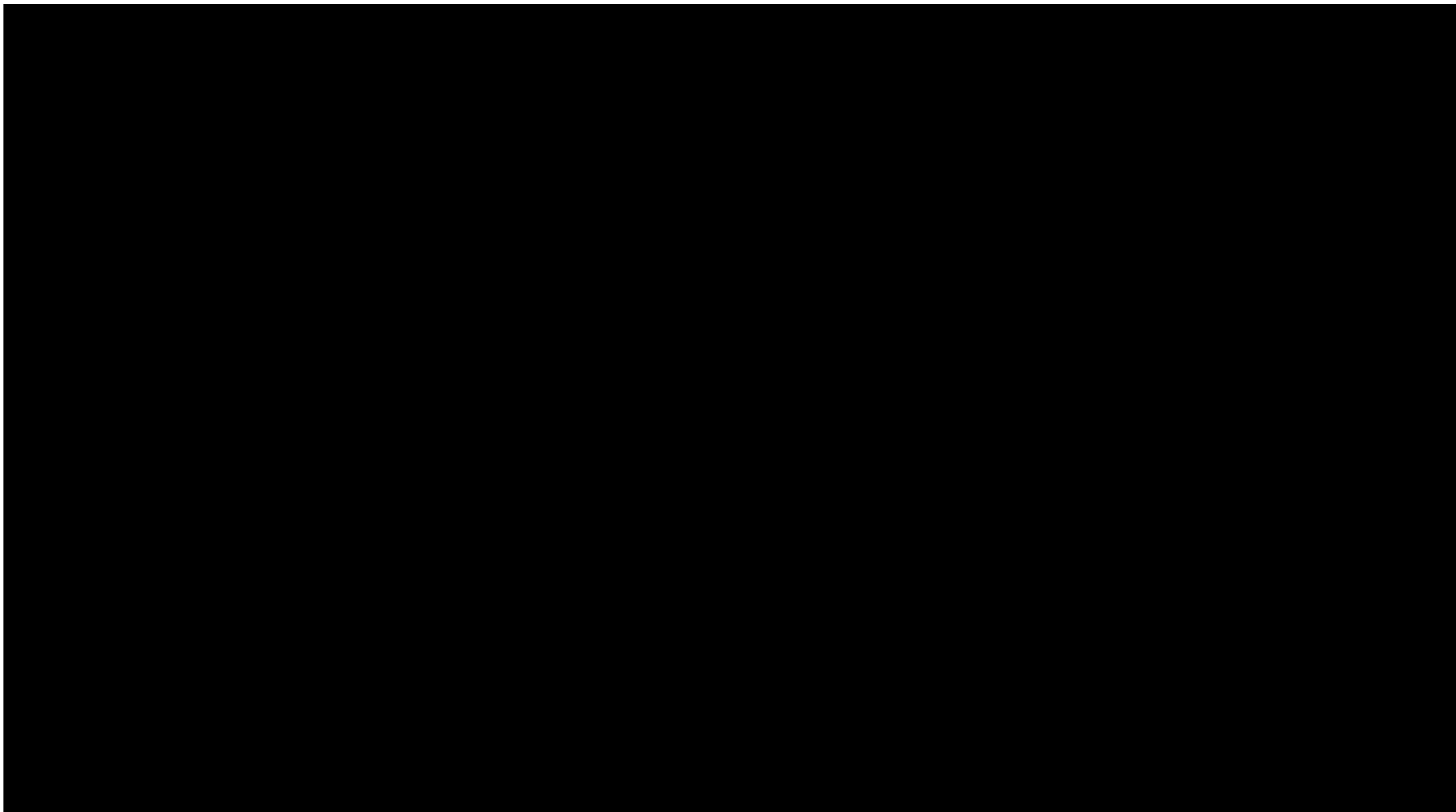


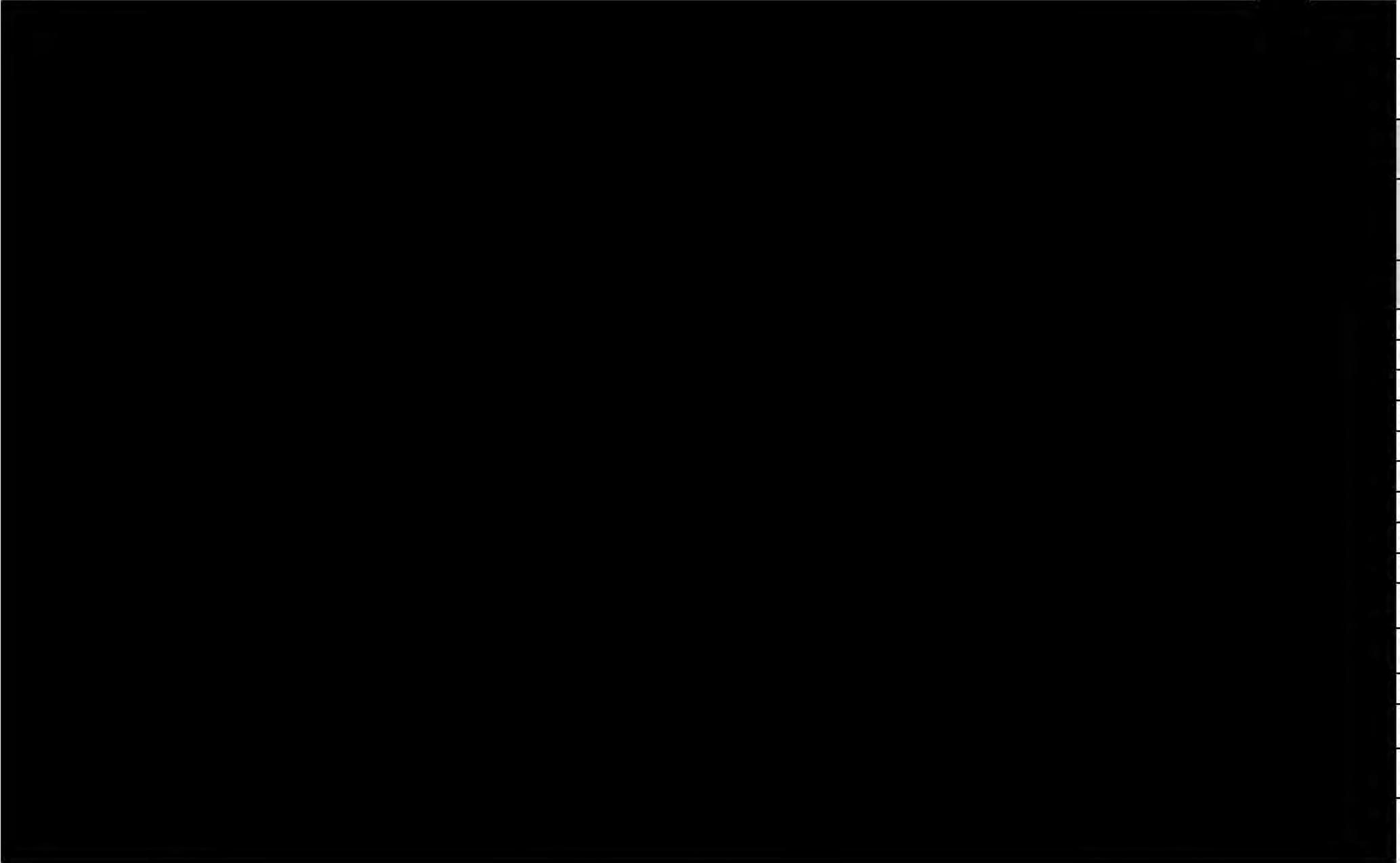
7 STUDY ASSESSMENTS AND PROCEDURES

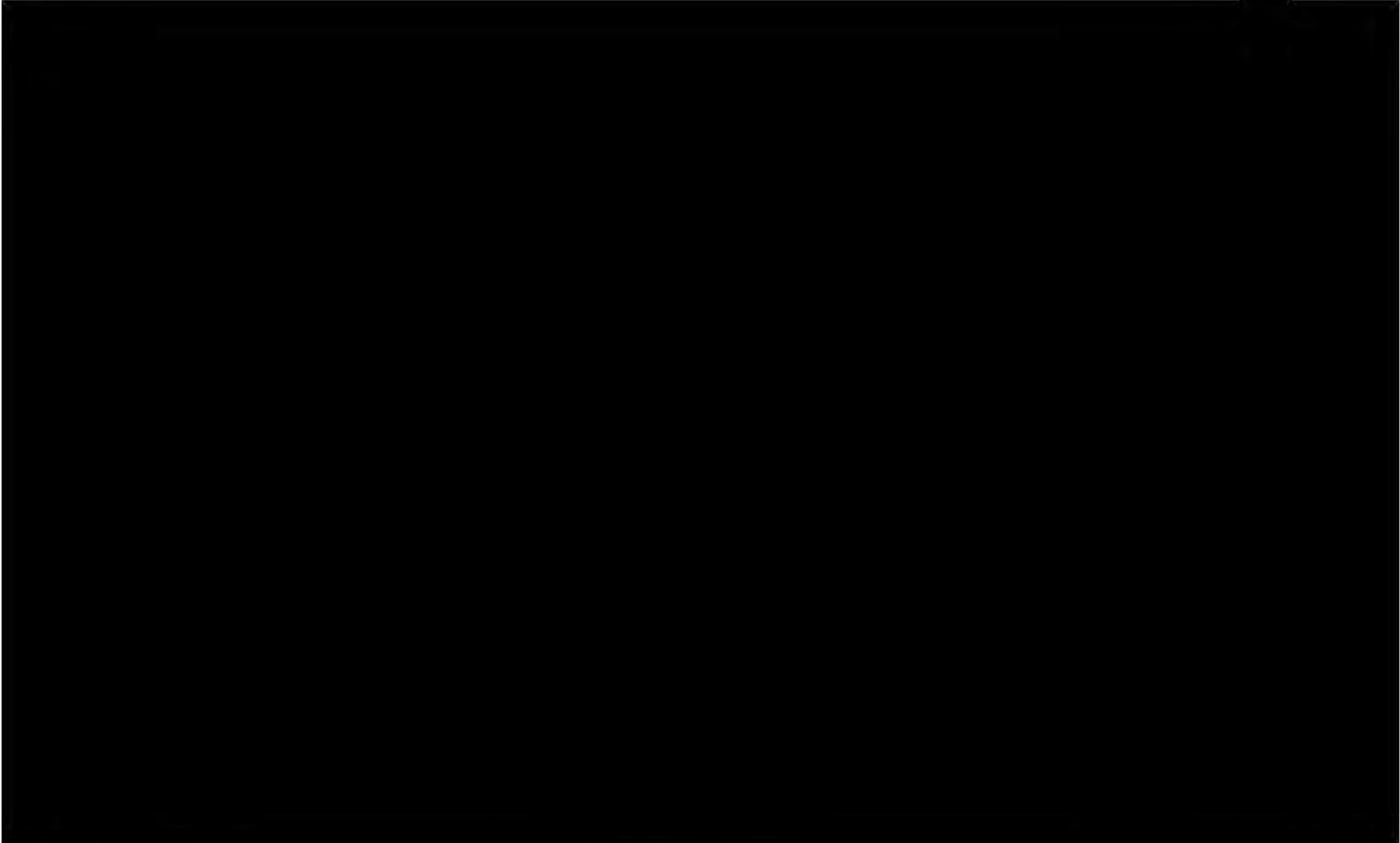
7.1 Study schedule

Study assessments and procedures, and their timings (including visit windows) are summarized in the Study Schedule ([Table 7](#)). 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.







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7.2 Screening period #1

The trial will include a 2-stage consent and screening procedure (see [Section 4.1](#)).

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 #1-6; 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 #2-4 (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 #6; 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).

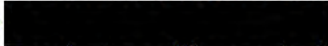
HLA-testing will be performed locally; information regarding the local lab, including methods used will be collected.

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  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 [REDACTED] 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); if systolic BP is below 100 mmHg or above 150 mmHg, and/or diastolic BP is below 50 mmHg or above 90 mmHg, the measurement will be



repeated; the HR measurement will be repeated when below 50 bpm or above 100 bpm,

- major body systems via physical examination; general appearance, skin, neck (incl. thyroid), ENT (ears, nose, throat), lungs, heart, abdomen, lymph nodes (all major stations palpable), extremities, neurology, and other sites as applicable (*i.e.* symptom directed),
- safety blood samples for hematology, coagulation, serum chemistry, urinalysis, hormones, and virus serology to be performed and evaluated locally, detailed as
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- for women of childbearing potential, highly sensitive serum pregnancy test,
- for postmenopausal women, FSH measurement for confirmation of postmenopausal status,
- a study kit to collect stools will be given to the patient at screening, and if the patient is eligible for the study, he/she will be asked to collect a stool sample any

time prior to [REDACTED] (if the patient fails screening, he/she will be asked to return the study kit to the site),

- if the individual patient has consented to the procedure, tissue samples will be collected; a maximum of [REDACTED], a [REDACTED], from the patient's tumor obtained at diagnosis or later during the disease (the newest available tissue sample would be preferred) - the samples will be sent to a central laboratory for analyses,
- PBMC and serum will be sampled; at each sampling time the target is to collect [REDACTED] for PBMC preparation, and [REDACTED] preparation - preparation/shipping procedures for the blood and serum samples will be detailed in the Laboratory Manual; a central laboratory will handle the immune response analyses,
- serum carcinoembryonic antigen (CEA) to be tested (local lab); *note, if an individual patient earlier during treatment of his/her disease has shown a positive result for another serum measurable tumor marker than CEA, this tumor marker should be followed in addition to CEA,*
- a computer tomography (with contrast) of chest, abdomen, and pelvis will be performed at screening (if not done within scope of normal care, not longer than [REDACTED] before the start of study treatment),
- 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

[REDACTED] are planned to be performed as outlined in Table 7, including visit windows of +/- 2 days if nothing else stated in Table 7 regarding specific items.

Study treatments are to be administered as outlined in Section 4, Section 6.4, and Section 6.5, and Table 7.

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 7), 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); if systolic BP is below 100 mmHg or above 150 mmHg, and/or diastolic BP is below 50 mmHg or above 90 mmHg, the measurement will be repeated; the HR measurement will be repeated when below 50 bpm or above 100 bpm,
- major body systems via physical examination; general appearance, skin, neck (incl. thyroid), ENT (ears, nose, throat), lungs, heart, abdomen, lymph nodes (all

major stations palpable), extremities, neurology, and other sites as applicable (*i.e.* symptom directed),

- 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 7](#)):

- at visits [REDACTED]
[REDACTED] assessment of TSH, and free T4 for evaluation of thyroid function, and assessment of serum cortisol for evaluation of potential adrenal insufficiency; if the outcome is pathologic further testing should be considered per [Section 7.3](#),
- for women of childbearing potential, a highly sensitive serum pregnancy test should be performed at visits [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED],
- a 12-lead ECG should be recorded according to local site standard procedures at visits V4, V8, and after this as medically indicated,
- a study kit to collect stools will be given to the patient at [REDACTED], and the patient will be asked to collect a stool sample any time prior to [REDACTED],
- tumor tissue to be collected at any time during study treatment when medically indicated procedure is done and it is possible to sample tissue, if the individual patient has consented to this procedure (the samples will be sent to a central laboratory for analyses as outlined in the objectives/endpoints of the trial; see [Section 3](#)).
- [REDACTED]
[REDACTED])
PBMC and serum will be sampled; at each sampling time the target is to collect [REDACTED]
[REDACTED]
- preparation/shipping procedures for the blood and serum samples will be detailed in the Laboratory Manual; a central laboratory will handle the immune response analyses,
- at visits [REDACTED] planned CEA tests should be done (see [Section 7.3](#) regarding possible additional serum tumor markers), and
- at visits [REDACTED] planned CT scans (see [Section 7.3](#) regarding possible additional serum tumor markers) should be done.

7.5 Post-treatment safety visits

Thirty days (-0/+4 days) after the last administration of trial treatment, a safety visit (Vd30) should be done for all patients, in addition the [REDACTED] should be [REDACTED] after the last treatment with nivolumab. 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 Vd30 [REDACTED]

- 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); if systolic BP is below 100 mmHg or above 150 mmHg, and/or diastolic BP is below 50 mmHg or above 90 mmHg, the measurement will be repeated; the HR measurement will be repeated when below 50 bpm or above 100 bpm,
- major body systems via physical examination; general appearance, skin, neck (incl. thyroid), ENT (ears, nose, throat), lungs, heart, abdomen, lymph nodes (all major stations palpable), extremities, neurology, and other sites as applicable (*i.e.* symptom directed),
- safety blood samples for hematology, coagulation, serum chemistry, urinalysis, TSH, free T4, serum cortisol, to be performed and evaluated locally (as detailed in [Section 7.3](#)),
- 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 disease progression

Patients who have permanently stopped all study treatment without having had confirmation of disease progression will be followed every 8 weeks +/- 4 days, starting 8 weeks after the latest CT investigation.

At these timepoints the following is to be done (see also [Table 7](#)):

- CT scanning is to be done [REDACTED] until documented disease progression (or until other anti-cancer treatment would be started before confirmed progressive disease),
- PBMC and serum will be sampled [REDACTED] until documented disease progression (or until other anti-cancer treatment would be started before confirmed progressive disease); at each sampling time the target is to collect 56 mL of blood for PBMC preparation, and 5 mL of blood for serum preparation -

preparation/shipping procedures for the blood and serum samples will be detailed in the Laboratory Manual; a central laboratory will handle the immune response analyses,

- serum CEA to be tested (local lab) [REDACTED] until documented disease progression (or until other anti-cancer treatment would be started before confirmed progressive disease); *note, if an individual patient earlier during treatment of his/her disease has shown a positive result for another serum measurable tumor marker than CEA, this tumor marker should be followed in addition to CEA,*
- documentation of safety; after the Vd30 and [REDACTED] for safety assessment have been made, only SAEs with plausible causal relationship with trial treatment will be documented (in addition, in case of death, an as precise cause of death as possible is going to be documented),
- if applicable, for female patients of childbearing potential, a serum pregnancy test will continue to be done on a monthly basis [REDACTED] 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.

7.6.2 Post-treatment visits after determination of disease progression

Patients who have permanently stopped all study treatment and have had confirmation of disease progression will be followed [REDACTED] after the time of determination of disease progression.

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 7](#)):

- assessment of survival status (if applicable, date of death, and cause of death, can be collected from local official registers or similar sources, *e.g.* records from other hospitals etc.),
- documentation of safety; after the Vd30 and [REDACTED] post-treatment visits for safety assessment have been made, only SAEs with plausible causal relationship with trial treatment will be documented (in addition, in case of death, an as precise cause of death as possible is going to be documented),
- if applicable, for female patients of childbearing potential, a serum pregnancy test will continue to be done on a monthly basis [REDACTED] 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 EO4010 in combination with nivolumab and/or with bevacizumab 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 (*Common Terminology Criteria for Adverse Events (CTCAE) v5.0 Quick Reference 8.5x11*, 2017).

The trial safety setup is described in [Section 4](#), 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 7](#).

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

Computed tomography scans will be performed according to the Study Schedule  for assessments of tumor status; when adequate due to *e.g.* location of new metastatic disease, other examinations than CTs might be necessary for documentation of tumor status - such assessments are going to be done, and recorded in the trial documentation, on an individual patient basis based on the judgment of the Investigator. Tumor response and progression in this trial will be evaluated using the revised international criteria (1.1) proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) committee ([Eisenhauer et al., 2009](#)) as well as the modified iRECIST guidelines ([Seymour et al., 2017](#)); see [Section 12.5](#) for a further description of the criteria. Investigators should especially note the different requirements for confirmatory scans as well as follow-up for the two criteria.

Of particular importance, in the context of confirmation of PD per iRECIST, and the given timeline of confirmatory scans to be performed at least 4 weeks, but no longer than 8 weeks after iUPD, is that in case of any signs of rapidly progressing disease establishment of PD and a following therapy switch should be made also earlier than 4-8 weeks after iUPD when necessary.

The main efficacy assessments, secondary endpoints of the trial, will include ORR, DCR, TTR, DOR, PFS, and OS as outlined in [Section 3.2.2](#).

7.9 Primary immunological assessments

7.9.1 Cell mediated immunity and associated utilization of PBMCs

Cell-mediated cytotoxicity is a key element in the proposed mechanism of action of the peptides composing EO4010. Results from various clinical trials, including different modes of immunizations, have shown the suitability of the IFN- γ ELISpot assay for monitoring T cell responses during immunizations. 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 by utilizing PBMCs in IFN- γ ELISpot assays, and by intracellular cytokine staining, and multimer staining. Samples will be collected at screening and during the trial according to [Table 7](#).

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 EO4010. Cross



reactivities with the corresponding nominal peptides from the targeted TAAs 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. Thus, PBMCs not utilized for immunogenicity measurements according to above might be used for RNA-sequencing of bulk and single cells to characterize factors as, for instance, T cell receptor-variation and immune status. In addition, collected PBMCs might also be used for assessment of further parameters related to induction of immunity, especially once an immune induction is indicated, *e.g.* cell killing potential, and tests related to antigen spreading.

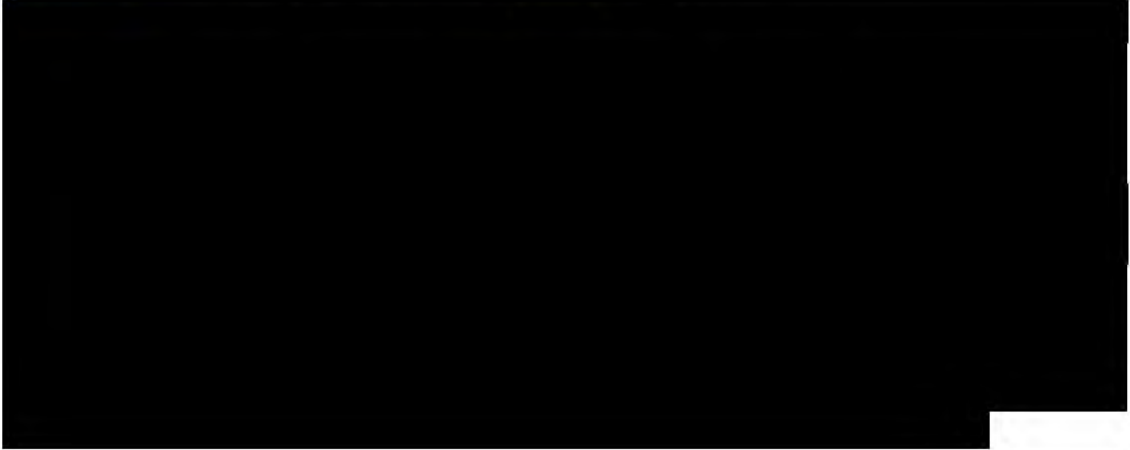
Preparation/shipping procedures for blood samples will be detailed in the Laboratory Manual; a central laboratory will handle the cellular immune response analyses.

7.9.2 Correlations between immunogenicity and other trial outcome parameters

As an exploratory objective, statistical assessments of correlations between immunogenicity (as measured by specific T cell responses) of the components of EO4010 (or their full-length counterparts), and efficacy and safety outcome parameters will be conducted. Cross reactivities shown for the nominal antigens 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:

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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 patient 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 EO4010 Investigator Brochure, or in the SmPC of nivolumab and in the SmPC bevacizumab, 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. In Europe, SUSARs will be reported as established in the EU Regulation No 536/2014; in the USA SUSARs will be reported as established in 21CFR312.32.

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 procedures). 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 means 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

In patients exposed to study treatment, the following must be reported from the date of the patient's signing the ICF until 6 months after the final administration of study treatment if no new anti-tumor treatment has been initiated earlier in which case the study related reporting will be terminated at the time of start of new anti-tumor treatment:

- pregnancy of the patient or the patient's sexual partner.

In addition, for consistency with nivolumab clinical trial protocols as advised by Bristol-Myers Squibb (marketing authorization holder for nivolumab), report all non-serious adverse events (not only those deemed to be treatment-related) continuously during nivolumab treatment periods and for a minimum of 100 days following discontinuation of nivolumab treatment.

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), patient lost to follow-up, 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 EO4010 monotherapy, or EO4010 in combination with nivolumab and/or bevacizumab, can be found in the current version of the EO4010 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 ([Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)).

Information about adverse reactions (causally related events) already known for bevacizumab can be found in the labelling documents for bevacizumab ([Zirabev | European Medicines Agency, 2023](#); [Zirabev-Drugs@FDA: FDA-Approved Drugs February 2023, n.d.](#))

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, EO4010 and nivolumab +/- bevacizumab are planned to be administered in combination, except for the two initial EO4010 administrations in Cohort 1, and therefore 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 EO4010, nivolumab and bevacizumab 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 ([Common Terminology Criteria for Adverse Events \(CTCAE\) v5.0 Quick Reference 8.5x11, 2017](#)); 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 7](#)).

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, *i.e.* Enterome (handled by Stragen Services SAS, 19 rue Jacqueline Auriol, 69008 Lyon, France; referred to as the Safety Group), 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 within the electronic data capture (EDC) system (eCRF-system) to the trial Safety Group within 24 hours of obtaining knowledge of the event.

The SAE Form (in the eCRF) 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. Once the AE



page has been updated in the eCRF for an SAE by the site, 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.

In case of technical or any other reason the SAE reporting cannot be carried out via the EDC system per above, the Investigator (or designated investigational site staff) will complete a paper SAE form and send to the Safety Group as outlined below. The paper SAE form will be provided to the sites prior to start of the study.

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

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

In case of urgent questions regarding SAE reporting please call:

[REDACTED]

[REDACTED]

[REDACTED]

[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. The Sponsor Safety Group will periodically request from the investigator targeted follow-up information using a specific Pregnancy Follow-up form.

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.

Note, in addition to the reporting of SAEs per above, the trial includes immediate reporting per below for all related Grade ≥ 3 adverse events (see also [Section 4.3](#)):

- *All related Grade ≥ 3 adverse events should be reported within 24 hours to the Sponsor via the Medical Monitor (reporting can be via the medical monitoring e-mail [redacted] or the medical monitoring telephone [redacted] and ALSO be reported as an SAE if it meets such criteria (see [Section 8.5.3](#)). Note, if the eCRF is filled out with the related Grade ≥ 3 adverse event within 24 hours this is also accepted as information to the Sponsor since there will be a special alert system implemented for such recordings in the eCRF.*

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. EO4010, trial EOCRC2-22),
- 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. The IDMC will examine the safety data of Cohort 1, including possible transitions between sub-cohorts (1a, 1b, 1c, and 1d) and provide advice and guidance in relation to the recruitment start of Cohort 2.

The major tasks for the IDMC are outlined in relation to the description of the design of the trial (see [Section 4](#)).

A key aspect of the IDMC work is related to assessments of the safety of individual patients developing related Grade ≥ 3 AEs. Notification of the IDMC of applicable such events is a key obligation of the Sponsor; the task is handled by the Sponsor's Medical Monitor of the trial who is immediately informed through an alert via the EDC system in all cases a Grade ≥ 3 AE (irrespective of relationship in a conservative oversight setup) is included in an eCRF for any patient. The Medical Monitor will also receive alerts, as the trial Safety Group do, when a SAE is generated within the EDC system.

In case of an alert the Medical Monitor will establish if there is a related Grade ≥ 3 AE, and if the IDMC should be notified (according to [Section 4](#)).

It is the Investigators, or designees, responsibility to inform the Sponsor's Medical Monitor if a patient encounters a related Grade ≥ 3 AE according to [Section 8.5.3](#).

The frequency of IDMC meetings will be based on the development of the safety profile of the study treatments and necessity to assess safety events and progression steps in the trial (e.g. transitions between cohorts).



The IDMC will also, if asked for by the Sponsor, meet in the case of patients' benefit from the study treatment at the time of planned study treatment discontinuation, or at the end of the trial, to advise the Sponsor of the management of such patients.

The IDMC members are to be international oncology/immune-oncology experts who are not in other ways than via the IDMC involved in the current trial. The IDMC will be described in detail in an IDMC Charter.

9 STATISTICAL EVALUATION

9.1 Analysis populations

The following analysis populations will be included:

- The All Patient 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 EO4010 and for whom no important protocol deviations occurred. See [Section 5.3.8](#) regarding replacement of patients.
- The Per-Protocol (PP) Population will consist of patients who received at least 4 doses of EO4010, for whom no important protocol deviations occurred, and who have at least one evaluable post-screening tumor assessment.
- The Safety Population will consist of patients who received at least one dose of EO4010. Patients who are not considered evaluable for this population will be replaced.
- The population evaluable for [REDACTED] in Cohort 1 will consist of patients who receive [REDACTED] of EO4010 and complete the [REDACTED] and patients who receive at least one dose of EO4010 but do not receive the second dose of EO4010 and/or do not complete the [REDACTED] due to safety reasons; *i.e.* in Cohort 1, if for reasons other than safety a patient does not receive [REDACTED] of EO4010 and complete the [REDACTED] the patient will not be counted towards [REDACTED]. Patients who are not evaluable for [REDACTED] in Cohort 1 will be replaced for [REDACTED] design assessment; however, if such a patient has received at least one dose of EO4010 he/she should be evaluated in the Safety Population.

Efficacy will be analyzed using both the FAS and PP populations. Safety will be analyzed using the Safety Population, and Cohort 1 will be analyzed based on patients evaluable for [REDACTED]

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. Objective response rate by the RECIST 1.1/iRECIST criteria will be estimated with two-sided exact 95% confidence intervals. TTR, DOR, PFS, and OS will be analyzed using the method of Kaplan-Meier and Cox proportional hazards model. The PFS rate at 4 months after the first dose of treatment, will be calculated based on all patients in the analysis set; the parameter will be considered important for comparisons with historical controls.

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 EO4010, 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.

9.4 Determination of sample size & primary analysis

This is an early development, open-label, exploratory, multi-cohort trial to include patients with previously treated metastatic CRC. In this context the sample size of the trial has been determined by practical considerations in collaboration with experts in the field, to facilitate an as fast as possible, and broad in Cohort 2 (in relation to patient baseline characteristics), assessment of safety and tolerability, immunogenicity, and preliminary efficacy. The sequential design of Cohorts 2 and 3, makes it possible to potentially, if needed based on trial data from Cohort 2, to adjust inclusion/exclusion criteria or other study parameters before start of recruitment of Cohort 3. Also, for efficient trial conduct it is assumed that an actual recruitment time must be kept under [REDACTED], *i.e.* [REDACTED] for Cohort 1 (safety lead-in; 3-12 evaluable patients), and then [REDACTED] each for Cohorts 2 (15 evaluable patients) and 3 (10 evaluable patients in 3a and 5 evaluable patients in 3b), respectively.

[REDACTED] for Cohort 1 include predefined stopping rules if concerning safety events would be encountered; in short 0 (zero) concerning safety event [REDACTED], or 1 concerning safety event in 6 patients, are considered acceptable.

The number of evaluable patients aimed at in Cohorts 2 and 3 (3a and 3b), *i.e.* 30 evaluable patients in total is considered to be relevant early dataset for safety assessments, and decision making regarding later stage development. From an efficacy perspective, by inference from earlier clinical experience in metastatic CRC, it is judged that there are at least two interesting outcome parameters to utilize for comparisons with historical groups, *i.e.* ORR (and DOR), and PFS rate [REDACTED]. The main benchmarking trials will be the phase 3 label enabling trials of trifluridine/tipiracil (TAS-102) and regorafenib. For regorafenib the following outcomes have been reported for the CORRECT phase 3 trial; median survival 6.4 months (95% CI, 5.8 to 7.3), median PFS 2.0 months (95% CI, 1.9 to 2.3), and ORR 1%. For trifluridine/tipiracil (TAS-102) the following outcomes have been reported for the RECURSE phase 3 trial; median survival 7.1 months (95% CI, 6.5 to 7.8), median PFS 2.0 months (95% CI, 1.9 to 2.1), ORR 1.6%, and DCR (CR, PR, and SD assessed at least 6 weeks after randomization) 44%. For TAS-102+ bevacizumab the following outcomes have been reported for the SUNLIGHT phase 3 trial; median survival 10.8 months (95% CI, to 9.4 to 11.8), median PFS 5.6 months (95% CI, 4.5 to 5.9), ORR 6.1%. For fruquintinib the following outcomes have been reported for the FRESCO-2 phase 3 trial; median survival 7.4 months, median PFS 3.7 months; ORR 2%.

If there would be at least two interesting outcomes in the current study within a group of 15 patients (which might be ORR, and prolonged PFS [REDACTED]) as judged by considering the above trial outcomes (*i.e.* at least at par with these trials), it could be considered to explore the treatment in further patients in a new trial (or via an amendment of the current trial).



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

Collection and storage of biological samples are described in the lab manual associated with EOCRC2-22 study. For more information, please refer to the lab manual.

10.5 Future use of patient samples; sample traceability

Following the completion of all study testing, the remaining tissue and blood/blood components may be used for future biomedical research (*e.g.* additional analysis on PBMC). This research will help to understand response against other tumor-associated antigens not included in the current peptide mix and possibly include DNA sequencing. If a patient requests destruction of his/her tissue and blood samples and the samples have not yet been de-identified, the Sponsor will destroy the samples under specific regulations and notify the Investigator in writing that the samples have been destroyed. Patients will be asked whether, or not they consent to the (optional) future use of patient samples. This consent will be documented separately, and patients will not be excluded from study participation if they do not consent to this future use of residual samples.

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.

10.6 Patient data protection

Patients will be assigned a unique identifier and will not be identified by name in eCRFs (data pseudonymization), 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.

10.7 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 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.

10.8 Handling of data and data collection

Data will be collected and processed in accordance with the General Data Protection Regulation (EU) 2016/679 and other national legislations as applicable. 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.

At an organizational level, to meet European requirements the sponsor has appointed a certified Data Protection Officer (DPO) in charge of ensuring compliance with data protection regulations. The DPO oversees the conduct of the required Data Protection Impact Assessment (DPIA) to assess and protect rights and freedoms of data subjects regarding processing operations. All data processors (e.g. sites and vendors) are bound by a data protection agreement (DPA). If personal data are transferred to a third country within the meaning of the GDPR, the DPA contains the Standard Contractual Clauses established by the European Commission. The DPO is also in charge of any data subject request and can be contacted directly by the data subject through the email provided in the information sheet.

At a technical level, personal data are stored in a secured environment, encrypted, and partitioned from other data. 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 trail 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. Entries made in the EDC screens should be completed within 2-4 days of the patient's study visit (see [Section 8.5](#) regarding timelines for immediately reportable events) and must be supported by source documents maintained for all patients enrolled in the study.

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.

10.9 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.

10.10 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.

10.11 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.

10.12 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.

10.13 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.

10.14 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.

Archiving of the clinical trial master file



Unless other law requires archiving for a longer period, the sponsor and the investigator shall archive the content of the clinical trial master file for at least 25 years after the end of the clinical trial.

10.15 Publications

The main data generated in trial EOCRC2-22/AUDREY 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, patient 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

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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) (*Common Terminology Criteria for Adverse Events (CTCAE) v5.0 Quick Reference 8.5x11*, 2017).

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, <i>e.g.</i> , 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 (*NYHA Classification & Stages* —, n.d.).

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 EOCRC2-22 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] (NIH Institutional Review Board Professional Administrators Committee Version 5.1, Regulatory Process Workgroup 11/18/2005, Protocol Deviations and Violations., n.d.)

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 ([Opdivo | European Medicines Agency, 2023](#); [Opdivo-Drugs@FDA: FDA-Approved Drugs October 2023, n.d.](#)). Systemic reaction at administration of EO4010 cannot be excluded either, even if not reported in the early development of the similar compound EO2401 (see [Section 1.2.5](#)).

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 antiinflammatory 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: Criteria for measurement of tumor response and progression; RECIST and iRECIST

Response and progression in this trial will be evaluated using the revised international criteria (1.1) proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) committee ([Eisenhauer et al., 2009](#)) as well as the modified iRECIST guidelines ([Seymour et al., 2017](#)). Investigators should especially note the different requirements for confirmatory scans as well as follow-up for the two criteria.

RECIST 1.1 Response and Evaluation Endpoints

Measurable Disease

Measurable tumor lesions (nodal, subcutaneous, lung parenchyma, solid organ metastases) are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with chest x-ray and as ≥ 10 mm with CT scan or clinical examination. Bone lesions are considered measurable only if assessed by CT scan and have an identifiable soft tissue component that meets these requirements (soft tissue component ≥ 10 mm by CT scan). Malignant lymph nodes must be ≥ 15 mm in the short axis to be considered measurable; only the short axis will be measured and followed. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters). Previously irradiated lesions are not considered measurable unless progression has been documented in the lesion.

Non-measurable Disease

All other lesions (or sites of disease), including small lesions are considered non-measurable disease. Bone lesions without a measurable soft tissue component, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, lymphangitic involvement of lung or skin and abdominal masses followed by clinical examination are all non-measurable. Lesions in previously irradiated areas are non-measurable, unless progression has been demonstrated.

Target Lesions

When more than one measurable tumor lesion is present at baseline all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. Note that pathological nodes must meet the criterion of a short axis of ≥ 15 mm by CT scan and only the short axis of these nodes will contribute to the baseline sum. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed. At baseline, the sum of the target lesions (longest diameter of tumor lesions plus short axis of lymph nodes: overall maximum of 5) is to be recorded.

After baseline, a value should be provided on the CRF for all identified target lesions for each assessment, even if very small. If extremely small and faint lesions cannot be accurately measured but are deemed to be present, a default value of 5 mm may be used. If lesions are too small to measure and indeed are believed to be absent, a default value of 0 mm may be used.



Non-target Lesions

All non-measurable lesions (or sites of disease) plus any measurable lesions over and above those listed as target lesions are considered non-target lesions. Measurements are not required but these lesions should be noted at baseline and should be followed as “present” or “absent”.

Response

All patients will have their BEST RESPONSE from the start of study treatment until the end of treatment classified as outlined below:

Complete Response (CR): disappearance of target and non-target lesions and normalization of tumor markers. Pathological lymph nodes must have short axis measures <10 mm (Note: continue to record the measurement even if <10 mm and considered CR). Residual lesions (other than nodes <10 mm) thought to be non-malignant should be further investigated (by cytology, specialized imaging, or other techniques as appropriate for individual cases) before CR can be accepted. Confirmation of response is only required in non-randomized studies.

Partial Response (PR): at least a 30% decrease in the sum of measures (longest diameter for tumor lesions and short axis measure for nodes) of target lesions, taking as reference the baseline sum of diameters. Non target lesions must be non-PD. Confirmation of response is only required in non-randomized studies.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD taking as reference the smallest sum of diameters on study.

Progressive Disease (PD): at least a 20% increase in the sum of diameters of measured lesions taking as references the smallest sum of diameters recorded on study (including baseline) AND an absolute increase of ≥ 5 mm. Appearance of new lesions will also constitute progressive disease (including lesions in previously unassessed areas). In exceptional circumstances, unequivocal progression of non-target disease may be accepted as evidence of disease progression, where the overall tumor burden has increased sufficiently to merit discontinuation of treatment or where the tumor burden appears to have increased by at least 73% in volume. Modest increases in the size of one or more non-target lesions are NOT considered unequivocal progression. If the evidence of PD is equivocal (target or non-target), treatment may continue until the next assessment, but if confirmed, the earlier date must be used.

Confirmatory measurement/duration of response

Confirmation: In non-randomized trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials. However, in all other circumstances, *i.e.* in randomized trials (phase II or III) or studies where stable disease or progression are the primary endpoints, confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.



Duration of overall response: The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study). The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment (in randomized trials, from date of randomization) until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD). The clinical relevance of the duration of stable disease varies in different studies and diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease. Note: The duration of response and stable disease as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency. The frequency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be taken into account if comparisons between trials are to be made.

**Table 8: Integration of target, non-target and new lesions into response assessment**

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Response for this Category also Requires
Target lesions ± non target lesions				
CR	CR	No	CR	Normalization of tumour markers, tumour nodes <10 mm
CR	Non-CR/Non-PD	No	PR	
CR	Not all evaluated	No	PR	
PR	Non-PD/ not all evaluated	No	PR	
SD	Non-PD/ not all evaluated	No	SD	Documented at least once ≥4 wks. from baseline
Not all evaluated	Non-PD	No	NE	
PD	Any	Any	PD	
Any	PD	Any	PD	
Any	Any	Yes	PD	
Non target lesions ONLY				
No Target	CR	No	CR	Normalization of tumour markers, tumour nodes <10 mm
No Target	Non-CR/non-PD	No	Non-CR/non-PD	
No Target	Not all evaluated	No	NE	
No Target	Unequivocal PD	Any	PD	
No Target	Any	Yes*	PD	
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “symptomatic deterioration”. This is a reason for stopping therapy, but is NOT objective PD. Every effort should be made to document the objective progression even after discontinuation of treatment. *Investigators should record all new lesions; if the new lesion is felt to be equivocal, treatment may be continued pending further assessments – see table 2.				

iRECIST Response Assessment

Overall response will also be assessed using iRECIST. Immunotherapeutics may result in infiltration of immune cells leading to transient increase in the size in malignant lesions, or undetectable lesions becoming detectable. The criteria are identical to those of RECIST 1.1 in many respects but have been adapted to account for instances where an increase in tumor burden, or the appearance of new lesions, does not reflect true tumor progression.

Key differences are described below. All responses defined using iRECIST criteria are designated with a prefix. iRECIST time-point and best overall responses will be recorded separately.

Confirming Progression

Unlike RECIST 1.1, iRECIST requires the confirmation of progression and uses the terms iUPD (unconfirmed progression) and iCPD (confirmed progression). Confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks after iUPD.



In the context of confirmation of PD per iRECIST, and the timeline of confirmatory scans to be performed at least 4 weeks, but no longer than 8 weeks after iUPD, it should be specifically noted that in case of any signs of rapidly progressing disease establishment of PD and a following therapy switch should be made also earlier than 4-8 weeks after iUPD when necessary.

iCPD is confirmed if further increase in tumor burden, compared to the last assessment, is seen as evidenced by one or more of the following:

- Continued increase in tumor burden (from iUPD) where RECIST 1.1 definitions of progression had been met (from nadir) in target, non-target disease or new lesions
 - Progression in target disease worsens with an increase of at least 5 mm in the absolute value of the sum
 - Continued unequivocal progression in non-target disease with an increase in tumor burden
 - Increase in size of previously identified new lesion (s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.
- RECIST 1.1 criteria are met in lesions types (target or non-target or new lesions) where progression was not previously identified, including the appearance of additional new lesions.

If iUPD is not confirmed at the next assessment, then the appropriate response will be assigned (iUPD if the criteria are still met, but no worsening, or iSD, iPR or iCR if those criteria are met compared to baseline). As can be seen in [Table 8](#), the prior documentation of iUPD does not preclude assigning iCR, iPR, or iSD in subsequent time-point assessments or as best overall response (BOR) providing that iCPD is not documented at the next assessment after iUPD.

New lesions

New lesions should be assessed and measured as they appear using RECIST 1.1 criteria (maximum of 5 lesions, no more than 2 per site, at least 10 mm in long axis (or 15 mm in short axis for nodal lesions), and recorded as New Lesions-Target (NLT) and New Lesion-Non-Target (NLNT) to allow clear differentiation from baseline target and non-target lesions.

New lesions may either meet the criteria of NLT or NLNT to drive iUPD (or iCPD). However, the measurements of target lesions should NOT be included in the sum of measures of original target lesions identified at baseline. Rather, these measurements will be collected on a separate table in the case record form.

PD is confirmed in the New Lesion category if the next imaging assessment, conducted at least 4 weeks (but not more than 8 weeks) after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT OR an increase (but not necessarily unequivocal increase) in the size of NLNT lesions OR the appearance of additional new lesions.

**Table 9: Time-point iRESPONSE**

Target Lesions*	Non-Target Lesions*	New Lesions*	Time Point Response	
			No prior iUPD**	Prior iUPD***; ***
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/Non-iUPD	No	iPR	iPR
iPR	Non-iCR/Non-iUPD	No	iPR	iPR
iSD	Non-iCR/Non-iUPD	No	iSD	iSD
iUPD with no change OR decrease from last TP	iUPD with no change OR decrease from last TP	Yes	NA	NLs confirms iCPD if NLs were previously identified and increase in size (≥ 5 mm in SOM for NLT or any increase for NLNT) or number. If no change in NLs (size or number) from last TP, remains iUPD
iSD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based in further increase in size of NT disease (need not meet RECIST 1.1 criteria for unequivocal PD)
iUPD	Non-iCR/Non-iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on: ○ further increase in SOM of at least 5 mm, otherwise remains iUPD
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: ○ previously identified T lesion iUPD SOM ≥ 5 mm and / or ○ NT lesion iUPD (prior assessment - need not be unequivocal PD)
iUPD	iUPD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: ○ previously identified T lesion iUPD ≥ 5 mm and / or ○ previously identified NT lesion iUPD (need not be unequivocal) and /or ○ size or number of new lesions previously identified
Non-iUPD/PD	Non-iUPD/PD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on ○ increase in size or number of new lesions previously identified
* Using RECIST 1.1 principles. If no PSPD occurs, RECIST 1.1 and iRECIST categories for CR, PR and SD would be the same. ** in any lesion category. *** previously identified in assessment immediately prior to this TP.				

All patients will have their iBOR from the start of study treatment until the end of treatment classified as outlined below.

**Table 10: iRECIST best overall response**

TPR1	TPR2	TPR3	TPR4	TPR5	iBOR
iCR	iCR, iPR, iUPD, NE	iCR, iPR, iUPD, NE	iUPD	iCPD	iCR
iUPD	iPR, iSD, NE	iCR	iCR, iPR, iSD, iUPD, NE	iCR, iPR, iSD, iUPD, iCPD, NE	iCR
iUPD	iPR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, NE, iCPD	iPR, iSD, iUPD, NE, iCPD	iPR
iUPD	iSD, NE	PR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, iCPD, NE	iPR
iUPD	iSD	iSD, iUPD, NE	iSD, iUPD, iCPD, NE	iSD, iUPD, iCPD, NE	iSD
iUPD	iCPD	Anything	Anything	Anything	iCPD
iUPD	iUPD	iCPD	Anything	Anything	iCPD
iUPD	NE	NE	NE	NE	iUPD

- Table assumes a randomised study where confirmation of CR or PR is not required.
- NE = not evaluable that cycle.
- Designation "I" for BOR can be used to indicate prior iUPD to aid in data interpretation.
- For patients with non-target disease only at baseline, only CR or non-CR/non-PD can be assigned at each TPR but is not shown in the table for ease of presentation.

Response and Stable Disease Duration (RECIST 1.1 and iRECIST)

Response duration will be measured from the time measurement criteria for CR/PR or iCR/iPR (whichever is first recorded) are first met until the first date that recurrent or progressive disease is objectively documented, taking as reference the smallest measurements recorded on study (including baseline).

Stable disease duration will be measured from the time of start of treatment until the criteria for progression are met, taking as reference the smallest sum on study (including baseline).

Methods of Measurement

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Assessments should be identified on a calendar schedule and should not be affected by delays in therapy. While on study, all lesions recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned. For lesions which fragment/split add together the longest diameters of the fragmented portions; for lesions which coalesce, measure the maximal longest diameter for the "merged lesion".

Clinical Lesions

Clinical lesions will only be considered measurable when they are superficial and ≥ 10 mm as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended. If feasible, imaging is preferred.

Chest X-ray

Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions ≥ 20 mm on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.



CT, MRI

CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans). Other specialized imaging or other techniques may also be appropriate for individual case.⁴ For example, while PET scans are not considered adequate to measure lesions, PET-CT scans may be used providing that the measures are obtained from the CT scan and the CT scan is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast).

Ultrasound

Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT is advised.

Endoscopy, Laparoscopy

The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor Markers

Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response.

Cytology, Histology

These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (*e.g.* with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is advised to differentiate between response or stable disease and progressive disease.



12.6 Appendix 6: 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.

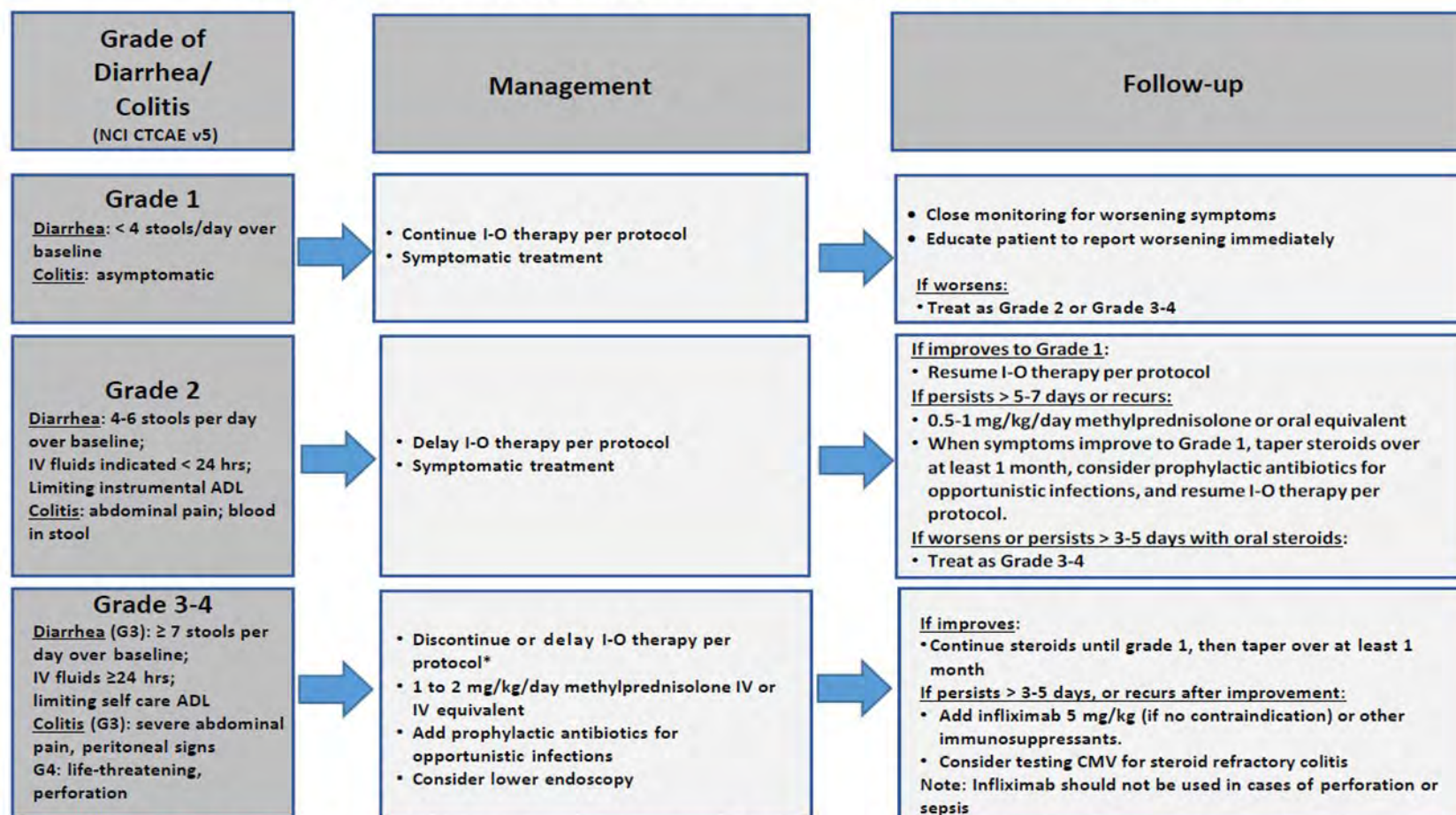
Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or procedure, is recommended.

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.



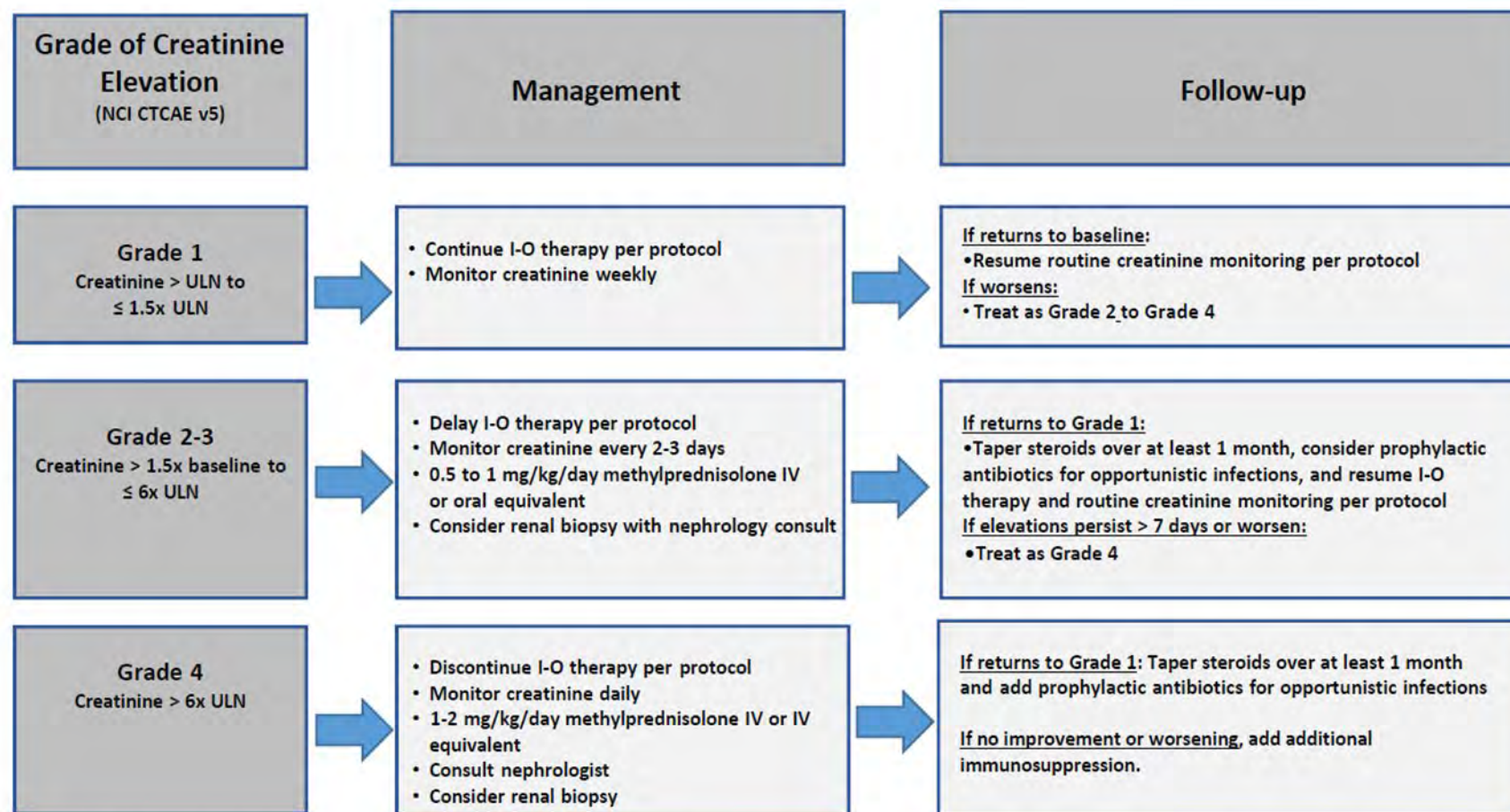
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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



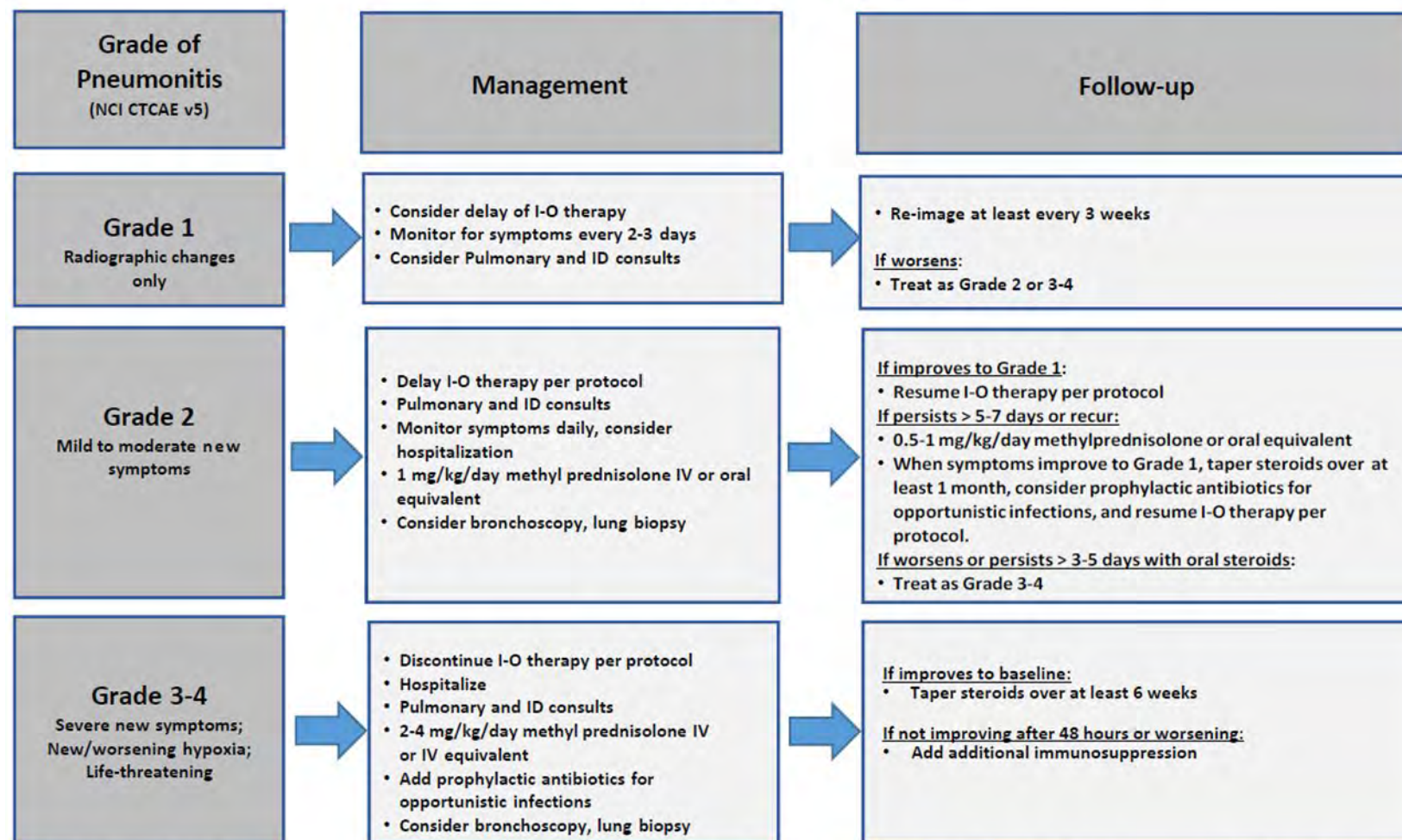
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

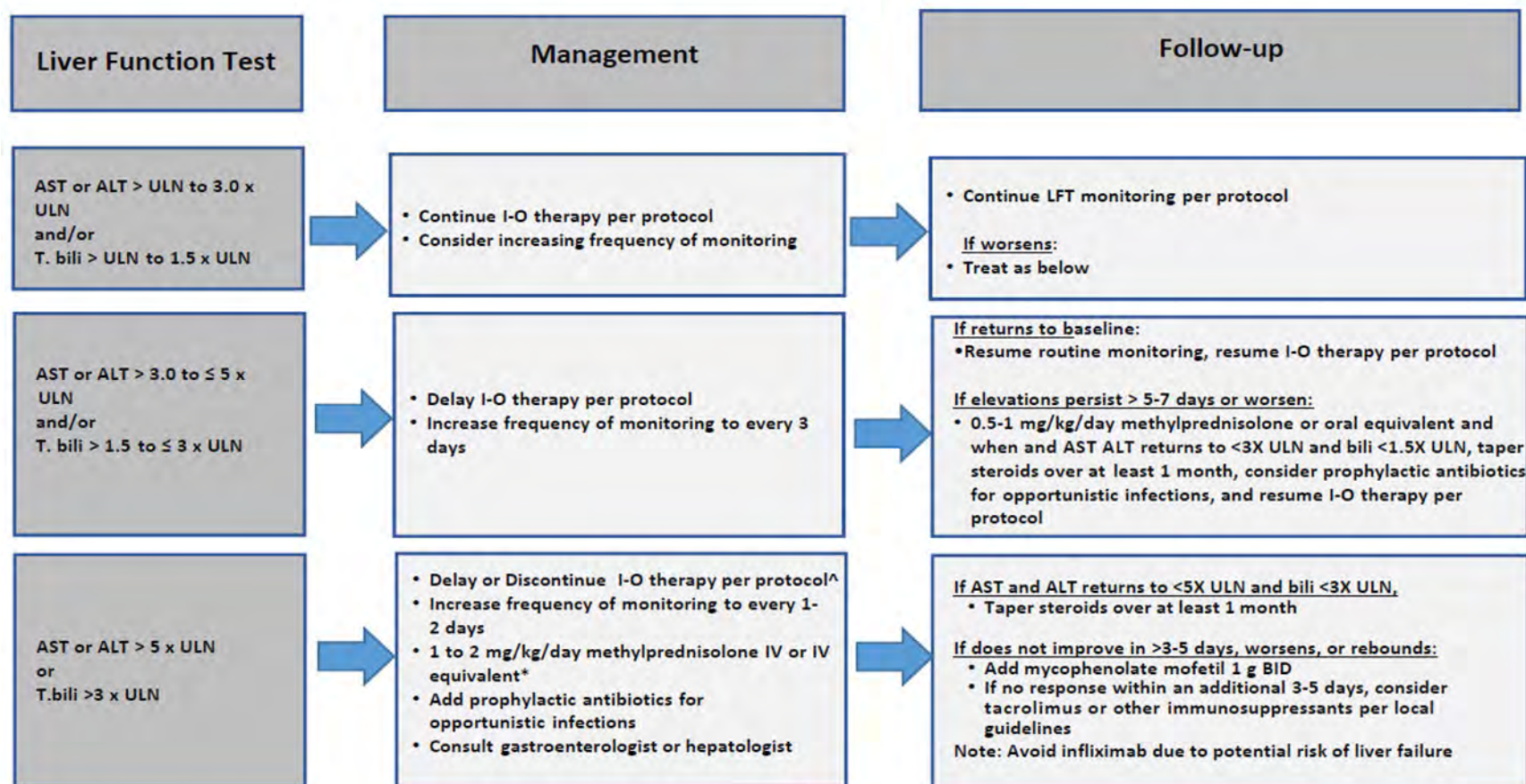


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

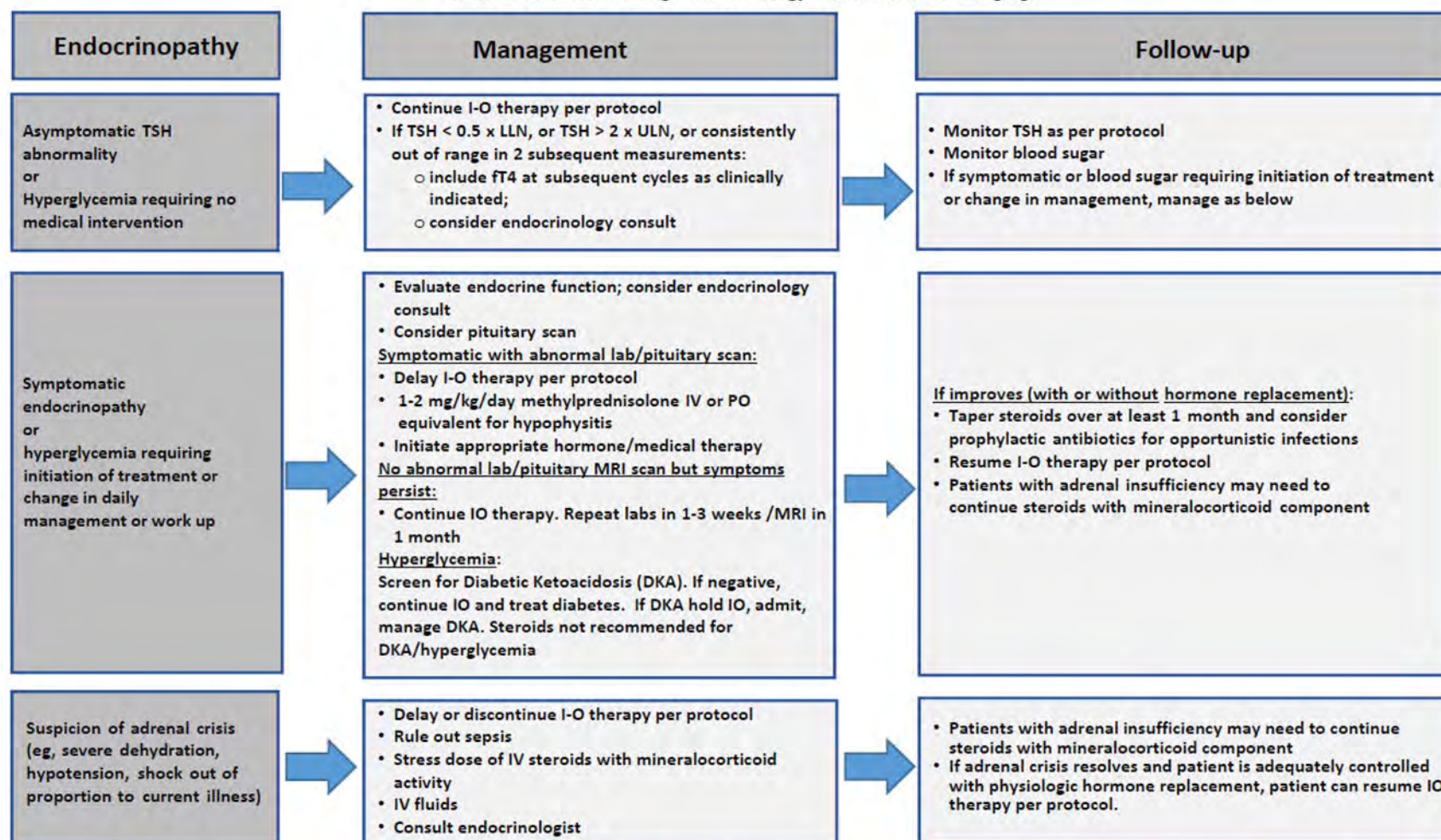
^Λ Please refer to protocol dose delay and discontinue criteria for specific details.

*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.



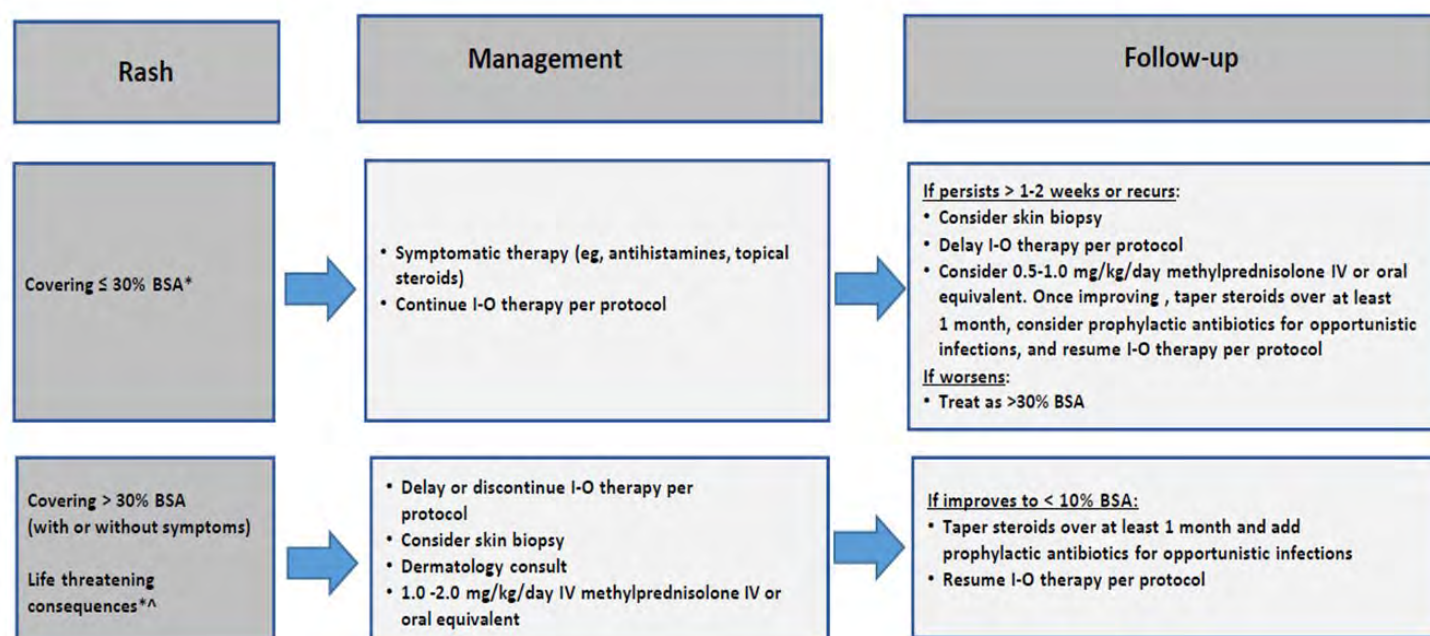
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg, prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

NOTE, below guidance is for nivolumab given as monotherapy! EO2401 administered with the adjuvant Montanide will lead to local administration site reaction in approximately 45-55% of patients, with a median duration of 6 weeks (see [Section 1.2.5.1](#)).

DO NOT FOLLOW THE BELOW GUIDANCE FOR ADMINISTRATION SITE REACTIONS RELATED TO EO4010; see SECTION 6.5.3 AND 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.



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.

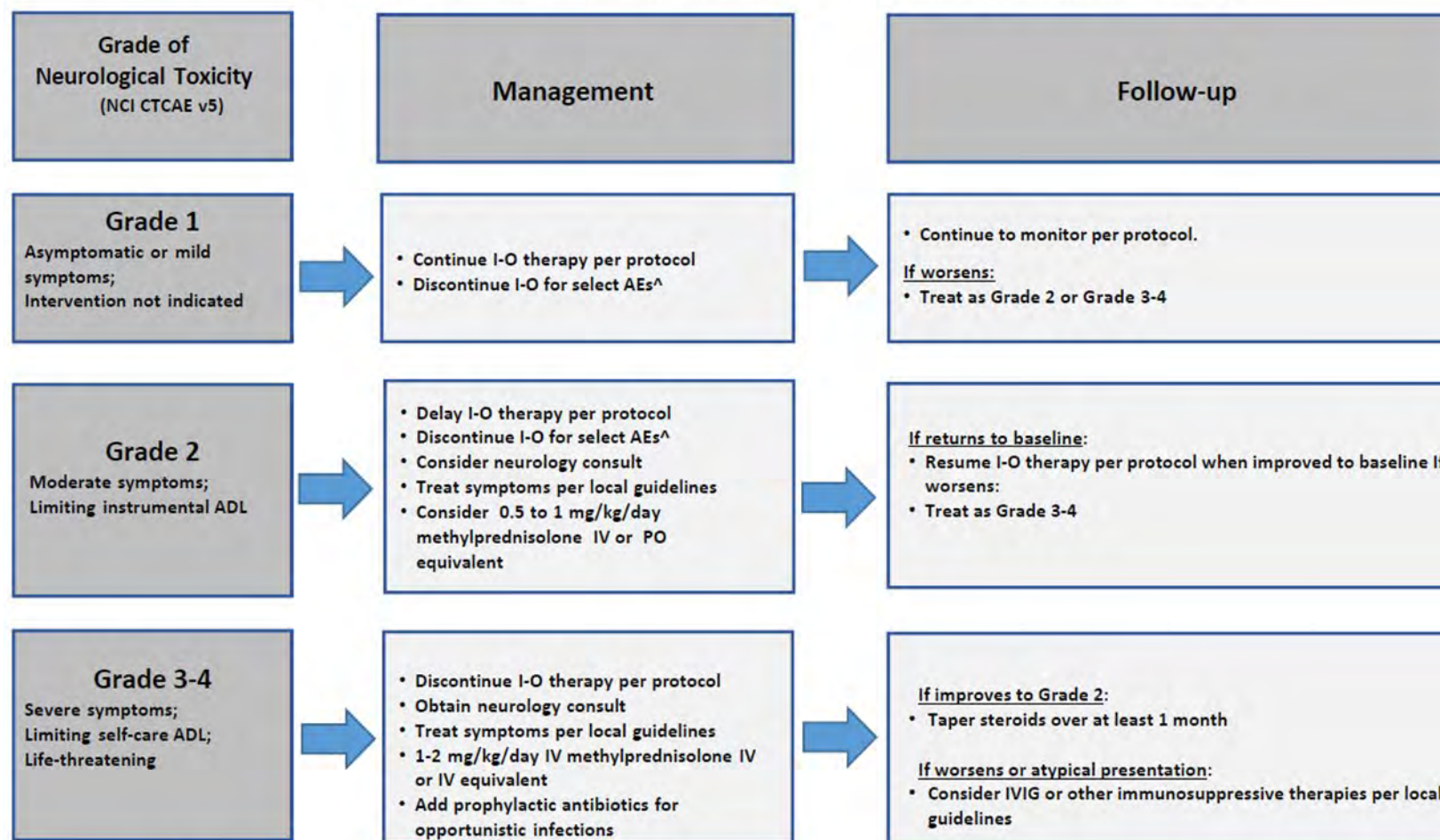
*Refer to NCI CTCAE v5 for term-specific grading criteria.

^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.



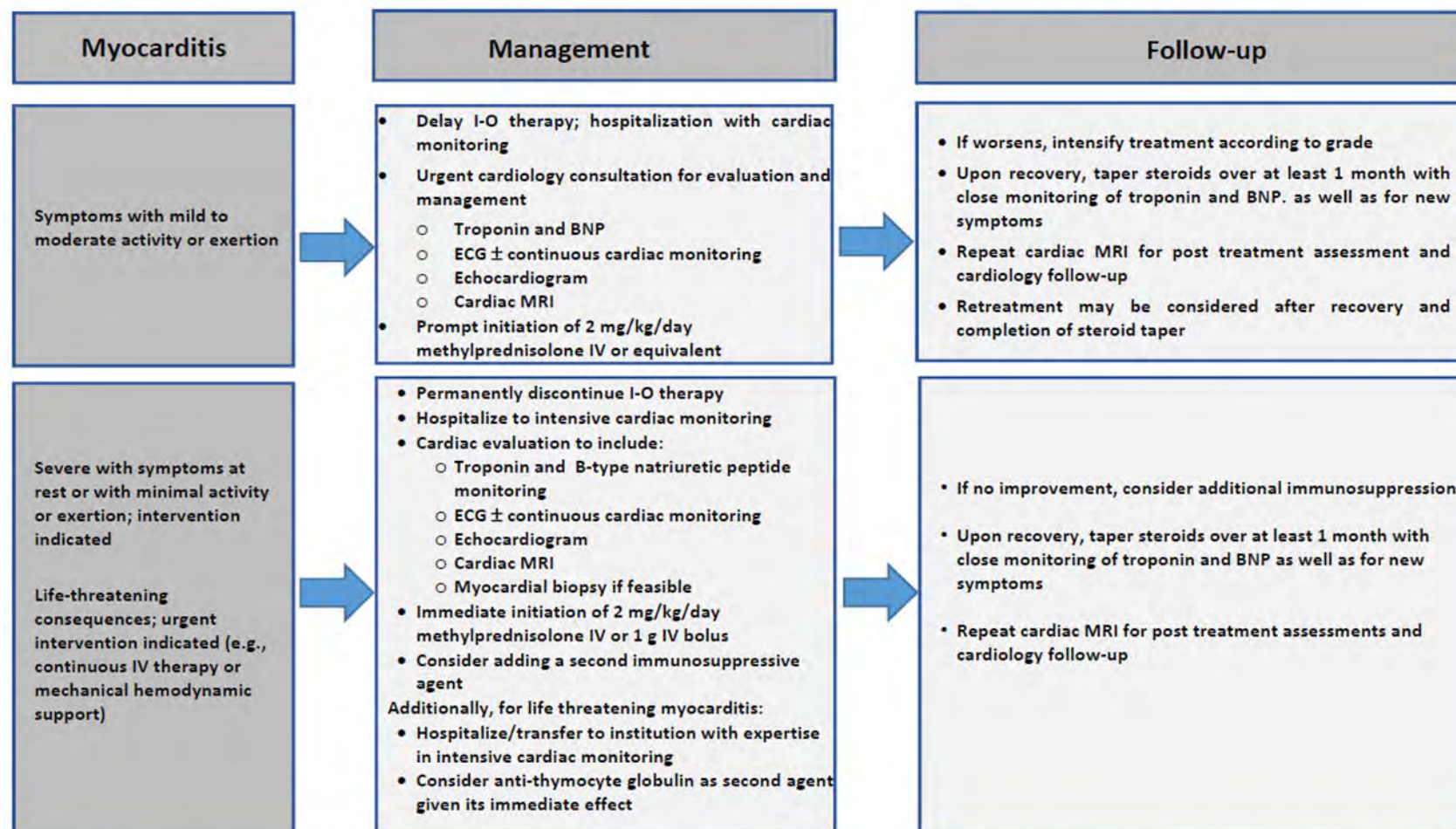
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (eg. prednisone) at start of tapering or earlier, after sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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