Protocol

A MulticenteR, Open-Label, First-in-Human, PhaSe Ib/IIa Trial of EO2401, a Novel Multipeptide Therapeutic VAccine, with and without PD-1 Check Point Inhibitor, FoLlowing Standard Treatment in PatIents with ProgrEssive Glioblastoma (Rosalie study)

Protocol Status: Final
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Investigational Product: EO2401

Protocol Reference Number: EOGBM1-18
EudraCT Number: 2018-002279-16

Sponsor:
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Chief Medical Officer

Medical Monitor:
[redacted]
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[redacted]

SPONSOR APPROVAL

I have read the following and approve it:

_____ _____

[redacted]
Chief Medical Officer

Date

Sponsor's information:
Rosalie is a musical with music by George Gershwin and Sigmund Romberg, lyrics by Ira Gershwin and P.G. Wodehouse.
It was first produced on Broadway in 1928 at the New Amsterdam Theatre.
The name of this study has been established in tribute of George Gershwin who died of glioblastoma in 1937: he was 38 years old.

GLOBAL COORDINATING INVESTIGATOR AGREEMENT

I have read the following protocol and agree to conduct the study as described herein.

_____ _____

[redacted] Date

Global Coordinating Investigator

USA COORDINATING INVESTIGATOR AGREEMENT

I have read the following protocol and agree to conduct the study as described herein.

_____ _____

[redacted]
Date

USA Coordinating Investigator

INVESTIGATOR AGREEMENT

I have read the following protocol and agree to conduct the study as described herein.

Name:
Principal Investigator

Date

SYNOPSIS

Title of study: A MulticenteR, Open-Label, First-in-Human, PhaSe Ib/IIa Trial of EO2401, a Novel Multipeptide Therapeutic VAccine, with and without PD-1 Check Point Inhibitor, FoLlowing Standard Treatment in PatIents with ProgrEssive Glioblastoma (Rosalie study)

Protocol Date: V4.0, 27 April, 2022

Indication: Progressive GB

Number of Investigators and study centers:

Approximately 10 Investigators in Europe and/or the United States.

Development phase: Ib/IIa

Objectives:

Primary objective

The primary objective of this first-in-human (FIH) trial of EO2401 is to evaluate the safety and tolerability of EO2401 as monotherapy and in combination with nivolumab, and nivolumab/bevacizumab in patients with progressive glioblastoma (GB). The safety and tolerability evaluation include assessments of different patient populations within the group of patients with recurrent GB: i) Cohorts 1a, 2a, and 3 evaluate patients with measurable disease, ii) Cohort 2b evaluates patients with non-measurable disease (after surgery of recurrent disease, i.e. adjuvant treatment), and iii) Cohort 2c evaluates a neoadjuvant/surgery/adjuvant treatment concept.

Secondary objectives

The secondary objectives are:

- The evaluation of survival
- The evaluation of tumor progression and response by magnetic resonance imaging (MRI) assessment using the Immunotherapy Response Assessment in Neuro-Oncology (iRANO) criteria
- To assess the immunogenicity of EO2316, EO2317, EO2318, and universal cancer peptide 2 (UCP2) that compose EO2401 [redacted]. Cross reactivities with the human tumor-associated antigens (TAAs) interleukin 13 receptor alpha-2 (IL-13Rα2), survivin also called baculoviral inhibitor of apoptosis repeat-containing 5 (BIRC5), and forkhead box M1 (FOXM1) will also be tested.

Exploratory objectives

- To assess the neurologic function assessed by the Neurologic Assessment in Neuro-Oncology scale
- To explore the correlation between immunogenicity of EO2316, EO2317, EO2318, and UCP2 that compose the EO2401 and tumor progression and response outcome parameters
- [redacted]
- To assess humoral immune responses toward EO2316, EO2317, EO2318, and UCP2 that compose the EO2401 [redacted]
- [redacted]

[redacted]

[redacted]

Methodology/study design:

Study rationale

As GB is the most lethal brain tumor and the prognosis for patients is very poor, there is an urgent medical need to develop new drugs that can be incorporated into the therapeutic care for GB patients. Enterome is developing an innovative cancer peptide therapeutic vaccine, EO2401, based on the homologies between TAAs and microbiome-derived peptides that will be administered alone and in combination with nivolumab, and nivolumab/bevacizumab to generate preliminary safety and efficacy data in patients with progressive GB. EO2401 is composed of 3 synthetic microbial-derived peptides targeting IL-13Rα2, BIRC5 (survivin), FOXM1, and a helper peptide UCP2. [redacted]

Study design

This is a multicenter, Phase Ib/IIa, FIH study to assess the safety, tolerability, immunogenicity, and preliminary efficacy of EO2401 in patients with unequivocal evidence of progressive or first recurrent GB confirmed by MRI as defined by the Response Assessment in Neuro-Oncology (RANO) criteria [redacted]

The FIH protocol is an open-label multi-cohort study, intended to recruit a maximum of approximately 100 evaluable patients in total. The design builds on a 3-by-3 approach for Cohort 1 (as study safety lead-in, before global amendment 2) and then by the global amendment 2 (leading to EOGBM1-18 version 3) the planned patient numbers per cohort are:

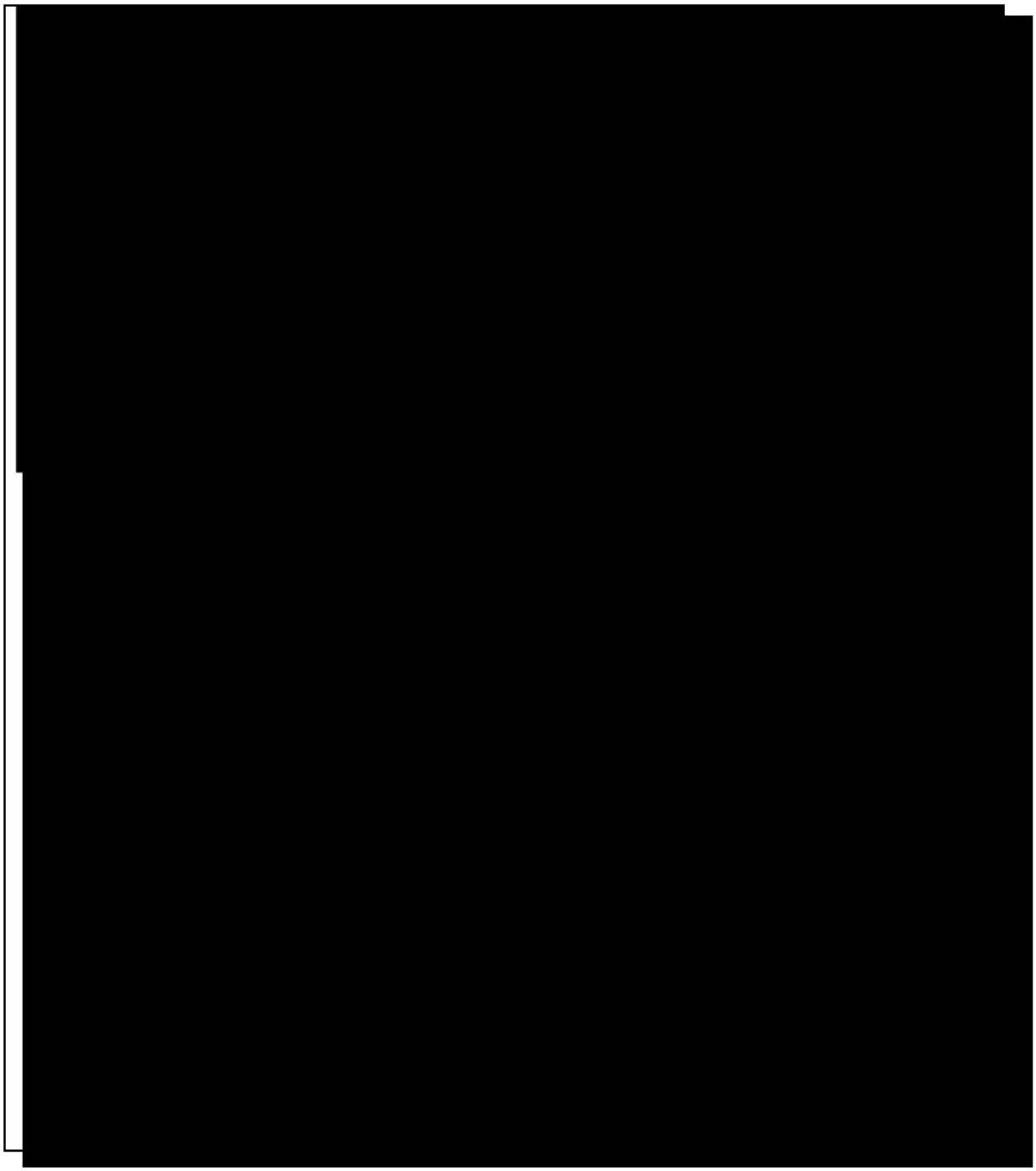
- Cohort 1a (sEN* treatment of patients with measurable disease): 3 patients in the safety lead-in part, plus 18 added patients by the global amendment 2 plus re-distribution of patients from Cohort 2b, also including extended patient management measures = total of 21 patients (recruitment finalized before global amendment 3)
- Cohort 2a (EN* treatment of patients with measurable disease): 23 patients before adding 15 further patients by the global amendment 2 plus re-distribution of patients from Cohort 2b, also including extended patient management measures = total of 38 patients (recruitment finalized before global amendment 3)
- Cohort 2b (EN* adjuvant treatment, no measurable disease): lowered from 15 patients to 6 patients by re-distribution to Cohorts 1a and 2a (recruitment finalized before global amendment 3), by the global amendment 2 also including extended patient management measures
- Cohort 2c (EN* neoadjuvant treatment - surgery - adjuvant treatment); cohort added by the global amendment 2 to evaluate safety and feasibility of the approach in 6 patients, also including extended patient management measures

- Cohort 3 (ENB* treatment of patients with measurable disease): by global amendment 3 target recruitment increased from 10 to 26 patients, by the global amendment 2 also including extended patient management measures

  **sEN = "slow" EO2401/nivolumab (delayed N for 4 weeks); EN = EO2401/nivolumab (combination treatment from start); ENB = EO2401/nivolumab/bevacizumab (bevacizumab full dose according to US label, from global amendment 3 recruiting at all sites and in all regions, not only recruiting in the US as initially during study conduct)*

The schematic study design with actual outcome of protocol versions 1 and 2, and Cohort Plan for version 3 are shown in the following schemes and details are provided in Section 3.1 of the protocol.

[redacted]



[redacted]

Number of patients:

It is anticipated that a maximum of approximately 100 patients evaluable for safety will be enrolled into the multi-cohort study.

There will be no formal sample size calculation for this study as this is an FIH trial.

Diagnosis and main criteria for inclusion and exclusion:

The following are the inclusion criteria:

1. Patients with unequivocal documented (including histological confirmation of GB at the primary diagnosis) first progression/recurrence of GB on MRI, as defined by RANO criteria
2. Patients with:
   - for Cohorts 1, 2a, and 3: at least 1 measurable lesion
   - for Cohort 2b: no measurable enhancing disease (defined as less than 1x1 cm in maximum bi-perpendicular plane)
   - for Cohort 2c: documented recurrence of GB deemed to be candidate for surgery as standard-of-care at the local institution, and for which the resection can safely be postponed for 4-6 weeks per local institutional guidance and treating physician judgement. In addition, for inclusion in Cohort 2c the patient must consent to mandatory collection of tissue samples from the time of diagnosis (if logistically available), and the planned surgery after neoadjuvant study therapy (see also **Section 7.1.1**).
     - [redacted]
3. Patients with an age ≥ 18 years old
4. Patients who are human leukocyte antigen (HLA)-A2 positive
5. Patients with an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 or Karnofsky performance status ≥ 70
6. Patients should have received standard primary therapy, including surgery (biopsy, incomplete or complete resection), radiation, temozolomide, if applicable
   a. Radiation therapy must have been finished at least 28 days before first study treatment administration
   b. Patients who received temozolomide as adjuvant therapy must have stopped the treatment and have a wash-out period of at least 28 days before first study treatment administration (6 weeks for nitrosoureas and at least 4 weeks, or 5 half-lives if longer, for experimental therapies, if this type of therapies have been included as components of adjuvant therapy)
   c. Patients with unmethylated methylguanine-DNA-methyltransferase (MGMT) promoter can be included even if they have not received temozolomide prior to the inclusion in this clinical study). [redacted]
7. Female patients of childbearing potential must have a negative serum pregnancy test within 72 hours prior to dosing
8. Considering the embryofetal toxicity of the nivolumab shown on 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 included:

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

iv. Intrauterine hormone-releasing system

v. Bilateral tubal occlusion

vi. Sexual abstinence.

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 must use condom from signing the ICF through 8 months after the last treatment dose administered. Males must ensure that their partners of childbearing potential use highly effective contraception also.

9. Patients having received the information sheets and who have provided written informed consent prior to any study-related procedures
10. Patients willing and able to comply with the scheduled visits, treatment plan, laboratory tests, and other study procedures indicated in the protocol.

The following are the exclusion criteria:

1. Patients treated with dexamethasone > 2 mg/day or equivalent (i.e., 13 mg/day of prednisone) within 14 days before the first EO2401 administration, unless required to treat an adverse event (AE)

   [redacted]

2. Patients treated with radiotherapy, and cytoreductive therapy within 28 days (6 weeks for nitrosoureas) before the first EO2401 administration. In addition, patients should not have received any prior treatment with compounds targeting PD-1, PD-L1, CTLA-4, or similar compounds where general resistance against therapeutic vaccination approaches might have developed; also, patients should not have received systemic anti-tumor treatment or radiotherapy for their progressive or first recurrent GB
3. Patients with tumors primarily located in the infra-tentorial segment
4. Patients with known radiological evidence of extracranial metastases
5. Patients with presence of new hemorrhage (excluding, stable Grade 1) or uncontrolled seizure
6. Patients with significant leptomeningeal disease

7. Patients with abnormal (≥ Grade 2 National Cancer Institute-Common Terminology Criteria for AEs [NCI-CTCAE] version 5.0) laboratory values for hematology, liver, and renal function (serum creatinine). In detail, the following values apply as exclusion criteria:
   [redacted]
8. For patients who are planned to receive bevacizumab:
   a. Patients with nephrotic syndrome
   b. Patients with proteinuria ≥ 2g/24 hours
   c. Patients with history or active gastrointestinal perforation and fistula
   d. Significant surgical procedure in the 4 weeks preceding the start of treatment or planned surgery
   e. Unhealed wound
   f. Patient with recent (4 weeks) history of hemoptysis of ½ teaspoon or more of red blood
   g. Thrombotic episode within 6 months
   h. Uncontrolled diabetes mellitus or hypertension
   i. Posterior reversible encephalopathy syndrome
9. Patients with persistent Grade 3 or 4 toxicities (according to NCI-CTCAE v5.0). Toxicities must be resolved since at least 2 weeks to Grade 1 or less. However, alopecia or other persisting toxicities Grade ≤ 2 not constituting a safety risk based on Investigator's judgment is acceptable
10. Patients with contraindication to contrast-enhanced MRI
11. Other malignancy or prior malignancy with a disease-free interval of less than 3 years except those treated with surgical intervention and an expected low likelihood of recurrence such as basal cell or squamous cell skin cancer, or carcinoma in situ. Patients with adequately treated basal cell or squamous cell skin cancer, or carcinoma in situ are eligible
12. Patients with clinically significant active infection, cardiac disease, significant medical or psychiatric disease/condition that, in the opinion of the Investigator, would interfere with the evaluation of EO2401 or interpretation of patient safety or study results or that would prohibit the understanding or rendering of informed consent (i.e. only consent able patients can be enrolled in the study) and compliance with the requirements of the protocol – including (but not limited to):
    a. Bacterial sepsis or other similarly severe infections
    b. New York Heart Association > Grade 2 congestive heart failure within 6 months prior to study entry
    c. Uncontrolled or significant cardiovascular disease, including:
        i. Myocardial infarction within 6 months prior to obtaining informed consent

ii. Uncontrolled/unstable angina within 6 months prior to obtaining informed consent
iii. Diagnosed or suspected congenital long QT syndrome
iv. Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes).

d. Stroke within 6 months prior obtaining informed consent
e. Concurrent neurodegenerative disease
f. Dementia or significantly altered mental status.

13. Patients with suspected autoimmune or active autoimmune disorder or known history of an autoimmune neurologic condition (e.g., Guillain-Barré syndrome); [redacted]
14. Patients with history of solid organ transplantation or hematopoietic stem cell transplantation
15. Patients with history or known presence of tuberculosis
16. Pregnant and breastfeeding patients
17. Patients with history or presence of human immunodeficiency virus and/or potentially active hepatitis B virus/hepatitis C virus
18. Patients who have received live or attenuated vaccine therapy used for prevention of infectious diseases including seasonal (influenza) vaccinations within 4 weeks of the first dose of study drug
19. Patients with a history of hypersensitivity to any excipient present in the pharmaceutical form of investigational medicinal product
20. Patients under treatment with immunostimulatory or immunosuppressive medications, including herbal remedies, or herbal remedies known to potentially interfere with major organ function
21. Patients with known drug and alcohol abuse
22. Patients with known or underlying medical or psychiatric condition that, in the Investigator's opinion, would make the administration of study drug hazardous to the patient or obscure the interpretation of toxicity determination or AEs
23. Patients who have received treatment with any other investigational agent, or participation in another clinical trial (clinical trial including active interventions are prohibited; participation in clinical trials for data collection purposes only are permitted) within 28 days prior to first study treatment administration and during the treatment period. Note, for investigational agents there should be a wash-out period of at least 28 days, or 5 half-lives if longer, before first study treatment administration
24. Patients deprived of their liberty or under protective custody or guardianship. [redacted]

Test products, dose, and mode of administration:

EO2401 test product will be reconstituted prior to administration.

In the priming phase of sub-cohorts 1a and 1b, patients will receive a subcutaneous (SC) injection of [redacted] of EO2401 (300 µg of each peptide corresponding to a total peptide dose of 1.2 mg [redacted] every 2 weeks for a total of 4 injections. In the priming phase of sub-cohorts 1c and 1d, patients will receive an SC injection of [redacted] EO2401, i.e., half the dose of EO2401; should this situation occur (i.e. treatment within sub-cohorts 1c/1d), patients who will be included in Cohorts 2 and 3 will also receive an SC injection of [redacted] EO2401.

The priming phase will be followed by a boost phase with 1 administration every month starting 4 weeks after the final priming injection and continued until confirmed tumor progression,

intolerable toxicity, death, or early termination of the study at the request of the Sponsor, where study treatment will be stopped and appropriate standard of care will be initiated by the Investigator.

EO2401 will be injected [redacted] from the start of the reconstitution.

EO2401 will be administered first followed by nivolumab, [redacted]

Nivolumab will be co-administered starting with the third priming injection in Cohort 1 and from the beginning of the priming phase in Cohorts 2a, 2b and 3. Nivolumab will be administered as an intravenous (IV) infusion at a dose of 3 mg/kg every 2 weeks.

In addition to the nivolumab, bevacizumab will be co-administered in patients included in Cohort 3, from the beginning of the priming phase, as an IV infusion at a dose of 10 mg/kg every 2 weeks in combination with EO2401 and nivolumab. Bevacizumab will be administered [redacted] after the start of nivolumab administration per local practice at the study site.

Duration of patient participation in study:

Each patient will participate in the study for a maximum of 24 months from the time of informed consent through final study contact. The recruitment period will be approximately 20 months.

Study populations:

The following analysis populations will be included for this study:

The **All Patient Population** will consist of any patient who signed informed consent including Screen failures.

Full Analysis Set (FAS) will consist of any patients who received at least 1 dose of study drug (EO2401 alone or EO2401 in combination with nivolumab, or nivolumab/bevacizumab) for whom no important protocol deviations occurred. Patients who are not considered evaluable for this population will not be replaced.

Safety Population will consist of any patients who received at least 1 dose of study drug (EO2401 alone or EO2401 in combination with nivolumab, or nivolumab/bevacizumab). Patients who are not considered evaluable for this population will be replaced.

Per-Protocol (PP) Population will consist of any patients who received [redacted] study drug (EO2401 alone or EO2401 in combination with nivolumab or nivolumab/bevacizumab) for whom no important protocol deviations occurred and have at least 1 evaluable post-Screening tumor assessment. Patients who are not considered evaluable for this population will not be replaced.

Efficacy will be analyzed [redacted]. Safety will be analyzed using the Safety Population.

Outcome measures:

Primary endpoint:

The primary endpoint includes the incidences of AEs, treatment-emergent AEs (TEAEs), serious AEs (SAEs), deaths, and laboratory abnormalities using the NCI-CTCAE v5.0.

Safety endpoints will be assessed by review of summaries of AEs (or TEAEs)/SAEs, unless otherwise stated. Adverse events will be categorized by system organ class and preferred term using the current Medical Dictionary for Regulatory Activities version and will be graded according to NCI-CTCAE v5.0.

Secondary endpoints:

The secondary endpoints are:

- Overall survival (OS), defined as the time interval from the date of first study treatment administration to the date of death due to any cause
- The tumor progression and response by MRI measurements every 8 weeks using the iRANO criteria evaluated by:
    - Progression-free Survival (PFS), using the iRANO criteria and defined as the time interval from the date of first study treatment administration to the date of first occurrence of progression or death from any cause, whichever occurs first

- Objective Response Rate (ORR), defined as the proportion of patients with complete response (CR) and partial response (PR) according to the iRANO criteria
- Disease Control Rate (DCR), defined as the proportion of patients with CR, PR, and Stable Disease (SD) according to the iRANO criteria
- Duration of Response (DoR), defined as the time interval from the date of first occurrence of CR or PR to the date of first documentation of disease progression or death from any cause, whichever occurs first.

- The immunogenicity of EO2316, EO2317, EO2318, and UCP2 that compose EO2401, [redacted]

[redacted]

Exploratory endpoints:

The exploratory endpoints will be:

- Neurologic function assessed by the NANO scale
- Correlation between immunogenicity of EO2316, EO2317, EO2318, and UCP2 that compose EO2401 and the outcome parameters PFS, ORR, DCR, DoR, and OS
- [redacted]
- Humoral immune responses toward EO2316, EO2317, EO2318, and UCP2 that compose EO2401 [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

Statistical methods:

All data collected in this study will be documented with the help of patient data listings and summary tables and figures for all demographic and baseline characteristics, medical history, efficacy, and safety variables.

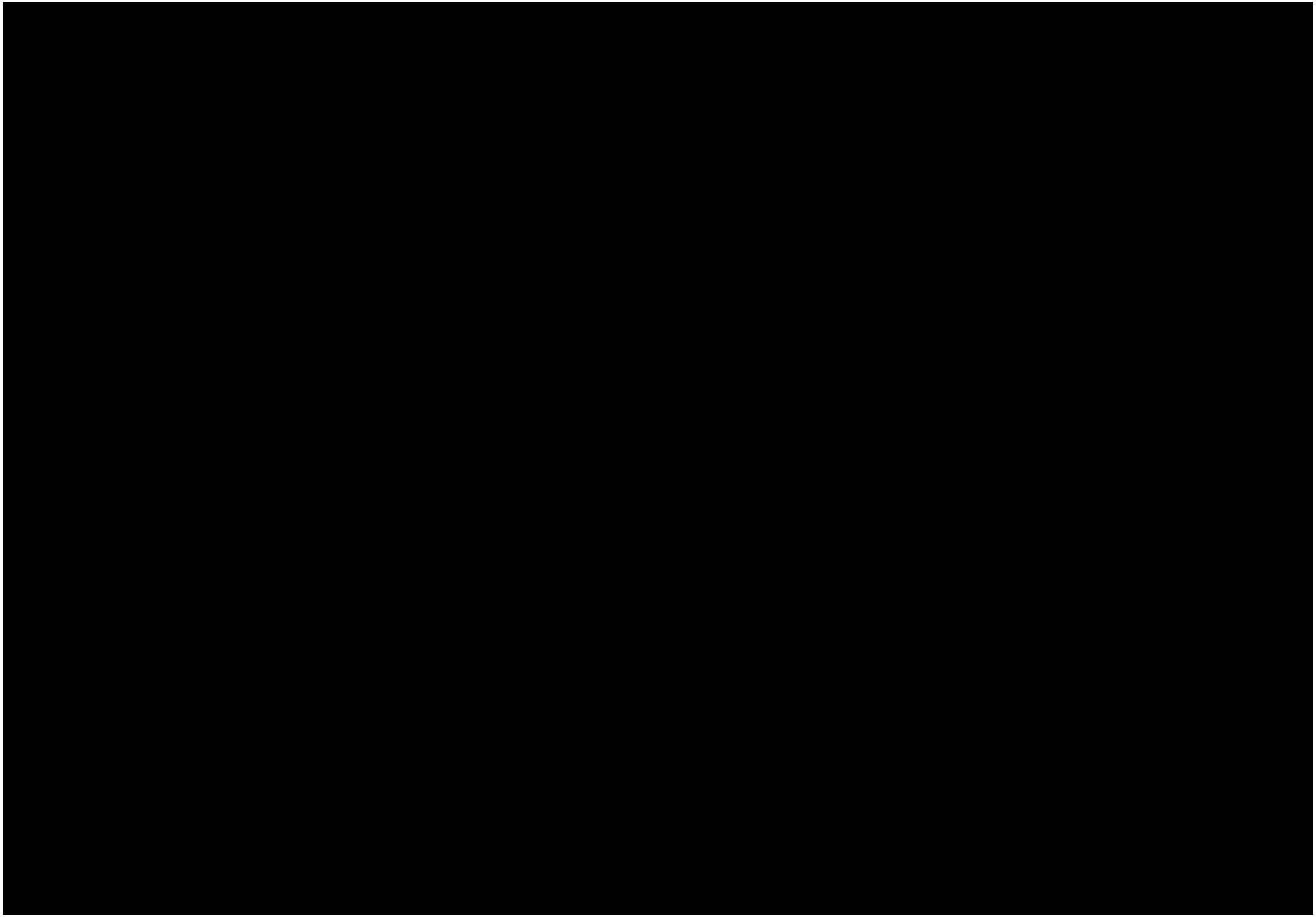
An interim analysis will be performed [redacted] to assess safety, immune response, and efficacy parameters.

[redacted]

Patients included in Cohort 2b will, depending on recruitment status and follow-up (targeted follow-up is 6 months), be analyzed in association with the first, and/or the second interim analyses.

[redacted]

[redacted]



[redacted]

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[redacted]

[redacted]

[redacted]

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ACTH	Adreno CorticoTrophic Hormone
AE(s)	Adverse event(s)
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
BIRC5	Baculoviral inhibitor of apoptosis repeat-containing 5
BP	Blood pressure
bpm	Beats per minute
CFR	Code of Federal Regulations
CI	Confidence interval
CNS	Central nervous system
ConA	Concanavalin A
CPI	Check Point Inhibitor
CR	Complete response
CRO	Contract Research Organization
CSA	Clinical study agreement
DCR	Disease Control Rate
DILI	Drug-induced liver injury
[redacted]	[redacted]
DoR	Duration of response
DSS	Drug Safety Services
[redacted]	[redacted]
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
EDC	Electronic data capture
[redacted]	[redacted]
[redacted]	[redacted]
EoT	End of treatment
FAS	Full Analysis Set
FIH	First-in-Human
FOXM1	Forkhead box M1
FSH	Follicle-stimulating hormone
GB	Glioblastoma
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HR	Heart rate
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceutical for Human Use
IDH	Isocitrate dehydrogenase
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IFA	Incomplete Freund's Adjuvant

Abbreviation	Definition
IFN-γ	Interferon-gamma
[redacted]	[redacted]
IL-13Rα2	Interleukin 13 receptor alpha-2
iRANO	Immunotherapy Response Assessment in Neuro-Oncology
IRB	Institutional Review Board
IMP	Investigational medicinal product
IV	Intravenous(ly)
MedDRA	Medical Dictionary for Regulatory Activities
MGMT	Methylguanine-DNA-methyltransferase
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
NANO	Neurologic Assessment in Neuro-Oncology
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
NTF	Note-to-File
ORR	Objective response rate
OS	Overall survival
P13K	Phosphatidyl inositol 3-kinase
[redacted]	[redacted]
PD	Progressive disease
PD-1	Programmed death-1
PD(L)-1	Programmed death (ligand)-1
PFS	Progression-free survival
PI	Product Information
PMEL	Pre-melanosome protein
PP	Per-Protocol
PR	Partial response
PsPD	Pseudoprogression
PT	Preferred term
PTHrP	Parathyroid hormone-related protein
RANO	Response Assessment in Neuro-Oncology
SAE(s)	Serious adverse event(s)
SC	Subcutaneous(ly)
SD	Stable disease
SmPC	Summary of Product Characteristics
SOC	System organ class
SUSAR(s)	Suspected unexpected serious adverse reaction(s)
TAA(s)	Tumor-associated antigen(s)
TEAE(s)	Treatment-emergent adverse event(s)
TERT	Telomerase reverse transcriptase
TCR	T cell receptor
TSH	Thyroid-stimulating hormone
UCP2	Universal cancer peptide 2
ULN	Upper limit of normal
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WBC(s)	White blood cell(s)
WHO	World Health Organization

1. INTRODUCTION

Glioblastoma (GB) is the most frequent and aggressive brain tumor in adults with a poor prognosis despite established standard treatment. Annual incidence ranges from 3 to 5 newly diagnosed cases per 100 000 populations. The median age at diagnosis is 64 years[1] and the incidence is slightly higher in men than women.

The management of patients with GB remains a challenge despite aggressive multimodal treatment. Current standard of care for newly diagnosed GB consists of resection followed by concurrent radiotherapy with concomitant temozolomide and adjuvant temozolomide.[2,3] However, despite initial treatment, about 70% of GB patients will experience disease progression within 1 year of diagnosis with less than 5% of patients surviving 5 years after diagnosis.[4]

The management of patients with progressive and recurrent disease outside of a clinical study will be individualized based on different factors, such as patient age, performance status, extension of the initial resection, and response to initial treatment. Repeat surgery, reirradiation, and second line mono or combination therapy are options in this patient population, but minor improvement in progression-free survival (PFS) and overall survival (OS) have been observed with any particular regimen.[2,3] Therefore, there is an urgent medical need to develop new drugs that can be incorporated into the therapeutic care for GB patients, given the opportunity to the patients to participate in experimental clinical studies.

The recent benefit observed with immunotherapy in the treatment of other tumor types, together with recent advances in the understanding of neuroimmunology, has offered new potential therapeutic options and interest for the development of immunotherapy for malignant brain cancer. Clinical studies using dendritic cell vaccines, peptide-based vaccines, and immune Check Point Inhibitors (CPIs) are underway. However, it is likely that combination regimens with complimentary mechanism of action will be required to achieve a broad and durable tumor response.[5]

1.1. Background on Disease

Glioma is a cancer originating from glial cells that occurs in the brain and spinal cord. The majority of primary gliomas (61%) are found in the 4 lobes of the brain.[4] Glial cells, the most common cell type found within the central nervous system (CNS), are the supportive cells of neurons. Gliomas are very aggressive, accounting for 70% of malignant brain tumor.[6] Although common among CNS cancers, gliomas are relatively rare with only 97 911 incident cases reported from 2008 to 2012 in the United States.[7] Gliomas are more common among Caucasians, and malignant gliomas are more commonly found in men compared to women.[8] Gliomas are graded based on 2 histological criteria – the resemblance of tumor cells to normal glial cells and the degree of the tumor malignancy. The grading system is developed by the American Brain Tumor Association.[9]

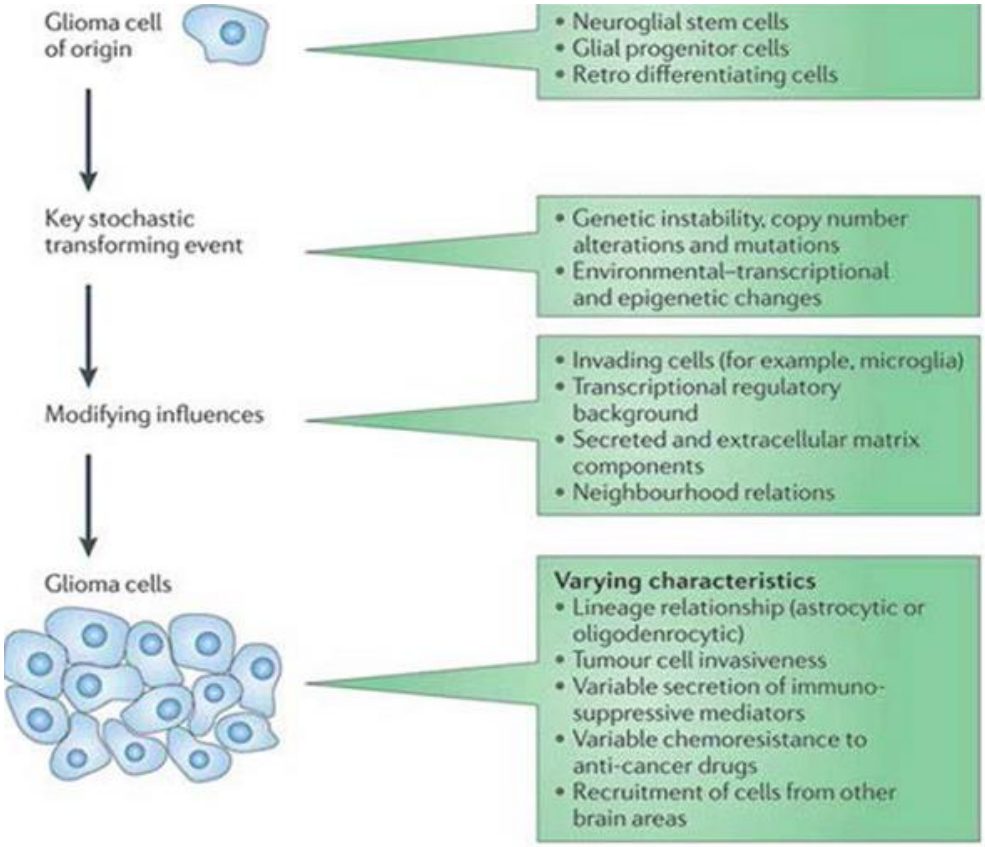
Diffuse astrocytomas (Grade II gliomas) are so named for their diffusive nature, spreading into nearby neural tissue. Although these tumors are diffuse, the cells typically have low to no mitotic activity so are considered “mid-grade” tumors. According to the most recent update of the World Health Organization (WHO) classification, they are characterized by an isocitrate dehydrogenase (IDH) mutation. These tumors are also typically treated using surgical resection, if possible, followed by radiochemotherapy.[10]

Anaplastic astrocytomas (Grade III gliomas) as well as anaplastic oligodendrogliomas and oligoastrocytomas are more malignant, and also actively spreading to other portions of the brain. These tumors are also characterized by IDH mutations, but there are also IDH wild-type subgroups. The differentiation between astrocytoma and oligodendroglioma is made based on 1p/19q deletion status. The median survival time for anaplastic astrocytomas is 2 to 5 years. These Grade III tumors comprise 20% to 25% of malignant gliomas. Approximately 10% of astrocytomas will progress to Grade IV.[11] Grade IV gliomas, also known as GBs, are the most common and most lethal of gliomas, with approximately 50% of gliomas being classified as GBs[12] and resulting in an average life expectancy of 12 to 15 months post-diagnosis.[6] Although there is marked variation in prognosis, symptoms, and response to treatment between the different types of gliomas, the morbidity and mortality are high, with even mild malignant types having 5-year survival rates below 50%.[8] Standard of care is radiation plus procarbazine, lomustine, and vincristine for oligodendroglioma with 1p/19q codeletion and radiotherapy plus maintenance temozolomide for anaplastic astrocytoma (without 1p/19q codeletion).[13]

The etiology of glioma has not been well established, but studies have demonstrated a correlation between glioma and several intrinsic and extrinsic factors. The only environmental agent that has been shown to strongly correlate with glioma is therapeutic ionizing radiation. Gliomas have been shown to develop within families, indicating a genetic component that partially contributes to susceptibility to disease. Grade IV gliomas (GB) have shown evidence of a genetic component through different genetic diseases that can give rise to GBs, including Turcot syndrome, tuberous sclerosis, neurofibromatosis type I, and multiple endocrine neoplasia type IIA. Certain acquired mutations have been associated with specific histological changes observed in glioma, where some have also been shown to be associated with low- or high-grade tumors.[8]

A schematic depicting various modifying steps and influences on development and progression of glioma is shown in Figure 1.

Figure 1: Sequential Events That Cause Glioma Diversity



Westphal and Lamszuz, 2011[14]

Glioblastoma, being the most common and the most lethal of the gliomas, is the most studied grade of glioma. Although morphologically and clinically indistinguishable, GB can manifest as either primary or secondary tumors. Approximately 90% to 95% of GB cases are considered primary, developing rapidly de novo in predominantly older patients without symptoms or evidence of low-grade pathology. Secondary GBs are rare and progress from low-grade diffuse gliomas like astrocytoma or anaplastic astrocytoma. Secondary GBs typically occur in younger patients and exhibit a much better prognosis, as compared to primary GBs.[12,14] Important genetic factors leading to the development of GB include inactivation of the p53 and retinoblastoma tumor suppressor pathways, dysregulation of growth factor signaling via amplification and mutational activation of phosphatidyl inositol 3-kinase (PI3K) genes, and activation of the PI3K pathway.[15] Histologically, GB is characterized by increased cellularity, necrosis, nuclear atypia, mitotic activity, vascular thrombosis, cellular pleomorphism, and glomeruloid microvascular proliferation, and clinically by its marked proliferative and invasive nature. [6,16,17] The prognosis for patients with GB is very poor. Median survival with a newly diagnosed GB is 12 to 15 months with current therapeutic interventions looking at study cohorts and even shorter if a real world population is considered. Twenty-five percent to 30% of patients survive up to 2 years and less than 5% of patients make it to a 5-year post-diagnosis survival.[18,19] There are no curative treatments currently available. The typical management of these tumors involves surgical resection as much as safely possible, radiotherapy, and concomitant and/or adjuvant chemotherapy with temozolomide.[20,21] However, disease recurs in almost all patients with the poor prognosis of 5 to 7 months of median survival. Current therapeutic modalities are insufficient to control high-grade glioma progression overall due to its aggressive nature. Although a variety of approaches, such as antiangiogenics[22] inhibitors of

the epidermal growth factor receptor, nitrosoureas, and retreatment with temozolomide, have been studied in the second line, there is not an established accepted standard therapy yet. Commonly, a second resection whenever feasible and also reirradiation are employed despite limited evidence (for resurgery)[13] and disappointing efficacy (second radiotherapy)[23]. Thus, it is an important medical need to continue the research in that setting and therefore, Enterome has selected to investigate EO2401 in the progressive GB population for the First-in-Human (FIH) study.

The symptoms of glioma vary between patients, depending on which subtype a patient has, as well as the physical size and location of tumor within the brain. Common symptoms across subtypes include headaches, nausea and vomiting, muscle weakness, seizures, confusion, dizziness, memory loss, and changes in personality.[6,24] Some of these more general symptoms can cause misdiagnosis due to their overlap with other infections, inflammatory diseases, and immunological diseases.[25] Focal symptoms map to more specific regions of the brain that are impacted by tumor growth, including loss of vision or other senses, motor weakness localized to one entire side of the body (hemiparesis), loss of the ability to understand or express speech (aphasia), and others.[20]

Diagnosis of glioma is done using various imaging techniques to visualize the brain tumor, including computed tomography scan, magnetic resonance imaging (MRI), positron emission tomography scan, and magnetic resonance spectroscopy.[21] Magnetic resonance imaging is the preferred and guideline appropriate imaging technique for diagnosing gliomas. Once the tumor has been visualized, the diagnosis is made by histopathological examination of the tumor through tumor resection (removing the entirety or majority of the visible tumor) or, when resection is not possible, through fine needle aspiration biopsy. It has to be acknowledged that the diffuse infiltrative, network-like growth pattern precludes a complete surgical resection and principal render WHO Grades II-IV gliomas a whole brain disease.

Novel approaches to treating high-grade gliomas are currently being investigated in clinical studies; however, only modest advancements in the treatment of GB have occurred in the past 25 years.[18,20,21]

In the last few decades, immunotherapy has become an important part of treating some types of cancer and is also being investigated to treat gliomas. Trials on checkpoint inhibition have been largely disappointing so far;[26] but better focus on risk groups or molecular factors may change these outcomes. Immunotherapies work by activating the immune system, causing it to specifically attack the tumor cells.[4] Tumor antigen-based vaccination represents such an approach that designed to enlist the patient's own immune system to recognize attack and destroy tumors, in a specific and durable manner.

Efficiency of this approach relies on the ability of the immune system to develop specific lymphocyte subsets able to recognize major histocompatibility complex (MHC) class I-restricted antigens that are expressed by human cancer cells.

Various tumor antigens have been studied for their potential to trigger immune-mediated responses against tumors. As a known example, interleukin 13 receptor alpha-2 (IL-13Rα2), survivin also called baculoviral inhibitor of apoptosis repeat-containing 5 (or BIRC5), or forkhead box M1 (FOXM1) are proposed targets for novel therapies in gliomas. These proteins, strongly upregulated in human glioma samples, are associated with disease progression and diminished survival in glioma patients.[27,28,29.] More recently, personalized approaches using peptides according to tumor-specific expression or mutation has gained a lot of attention and

examples like NEOVAC and GAPVAC convincingly demonstrated feasibility of tumor antigen-specific CD4 and CD8 responses in the tumor tissue.

1.2. Study Rationale

Currently, therapeutic modalities are insufficient to control high-grade glioma progression overall due to its aggressive nature. Surgical resection, as stated previously, is not always possible, and regrowth of tumors as well as acute morbidity is likely. As already indicated, radiotherapy has high toxicity, and retreating recurrences carries a higher risk due to loss in neurogeneration potential. Although chemotherapy has been shown to improve prognosis and time of progression, there are important drawbacks to this treatment modality. The major issues include bypassing the blood-brain barrier, interactions with antiseizure medications, resistance to therapy, recurrent tumors, and intrinsic factors.[30]

Enterome is developing an innovative, microbiome-based approach for the development of therapeutic cancer vaccines. EO2401 is a multicomponent therapeutic synthetic peptide vaccine that will be administered as a monotherapy and in combination with nivolumab or nivolumab/bevacizumab to assess the safety and tolerability, and generate preliminary efficacy data in patients with progressive GB.

1.2.1. Investigational Medicinal Product

1.2.1.1. EO2401

Peptide-based therapeutic vaccination is 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 to induce efficient presentation of cancer epitopes to T lymphocytes that in turn leads to a sustained immune response against cancer cells expressing the same antigens at the tumor site. In the past, despite promising preclinical results in animal models, the cancer vaccination approach has not demonstrated unequivocal efficacy in patients. The lack of efficacy may be related to a number of 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 tumor's ability to escape immune-mediated inhibition. The ability of tumor antigens to generate a strong immune response depends on a number of factors, including the affinity for the MHC I or MHC 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 are able to be efficiently reactivated by a vaccine boost and that leads to a durable immune response.

Enterome is developing an innovative, microbiome-based approach for the development of therapeutic peptide cancer vaccines. A number of peptides mimicking specific TAAs that are overexpressed in GB have been identified in the human microbiome. These peptides have been shown to induce a strong immune response in nonclinical models. Although overexpressed TAAs are presented via the MHC class I receptors, 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 some 70% of all T cells reside, is tolerant to the equivalent microbiome-derived epitopes. This "tolerance" can be overcome by repeated antigen challenges together with an adjuvant.

It has thus been demonstrated that the "general" human population has generated a "memory" repertoire of tolerized T cells recognizing the peptides from the microbiome.[31] These T cells are surprisingly abundant, can be circulating, and display memory phenotypes, all properties that can make them ideal to be studied in a vaccination protocol. The strategy of Enterome is thus to identify and then use these peptides to reactivate their associated memory T cells. Because the peptides are almost identical to known TAAs, they will activate memory T cells that will cross-react with the TAAs, thus inducing a strong attack against the tumor cells themselves. This approach as well as the identity and characteristics of the carefully chosen TAAs will be detailed further below in the relevant sections.

Thus, [redacted] microbiome-derived peptides that mimic specific TAAs that are overexpressed in GB have been identified (see Investigator's Brochure regarding expression of selected epitopes in GB and the high prevalence of the microbiome-derived peptide). These peptides were shown to induce a strong immune response in nonclinical models and are the specific ingredient part of the EO2401.

Before identifying these peptides, proof-of-concept studies were achieved with [redacted] microbiome-derived peptide with homology to a human IL-13Rα2 peptide. EO2315 elicited a strong binding affinity to the human leukocyte antigen (HLA)-A2. This peptide drove a strong immunogenicity in nonclinical models (HLA-A2 transgenic mice) and T cells generated against [redacted] demonstrated cross reactivity against the human peptide counterpart. The ability of [redacted] to promote cytotoxic T cell expansion in combination with anti-programmed cell death-1 was demonstrated in vivo using adoptive transfer of lymphocytes from immunized HLA-A2 transgenic mice into tumor-engrafted nude mice. Helper peptide was shown to improve the immunogenicity driven by [redacted]

Additionally, the immunogenicity of the universal cancer peptide 2 (UCP2) CD4+ helper peptide was demonstrated in the HLA-A2 transgenic mouse model with [redacted] and shown to improve the immunogenicity driven by [redacted].

Since then, [redacted] bacterial peptide sharing 8-amino acid with [redacted], was identified. While [redacted] shares the same immunogenic properties in mice as [redacted], [redacted] has been selected as a preferred candidate for EO2401 in comparison with [redacted] because of higher prevalence of [redacted] within the human gut microbiota.

EO2401 DP

EO2401 DP (not adjuvanted) is a [redacted] peptide mixture solution of 1 synthetic [redacted] microbiome-derived peptide (EO2316), and 2 synthetic [redacted] microbiome-derived peptides (EO2317 and EO2318), and the synthetic [redacted] UCP2 (CD4+ helper) peptide. EO2316, EO2317, and EO2318 were found in the human microbiota and display high homology with known and validated GB markers, the TAAs. A total of 3 microbiome-derived peptides that mimic 3 different TAAs expressed in GB were selected to overcome possible tumor heterogeneity and reduce tumor escape. All 3 peptides demonstrated high MHC binding affinity, strong immune responses as well as cross reactivity against the human corresponding peptides in nonclinical models. The multicomponent solution EO2401 DP [redacted]. The final concentration of each peptide is 300 µg/mL.

1.2.1.1.1. EO2316

EO2316 is [redacted] bacterial peptide that shows high homology to a cognate epitope on human IL-13Rα2 that is known as a decoy receptor for IL-13. IL-13Rα2 is an inhibitory subunit of the type II receptor. Normal function of IL-13 is to activate STAT6, which exerts transcriptional control over genes promoting apoptosis through increased caspase-3 activity. Sequestration of IL-13 by IL-13Rα2 is considered to be an apoptosis escape mechanism of tumor cells. In addition, IL-13Rα2 acts as a receptor for the chitinase-like protein Chi3l1 and mediates signaling leading to activation of mitogen-activated protein kinase/ERK, AKT, and Wnt/β-catenin pathways, all involved in the process of tumorigenesis. The link between IL-13Rα2 and tumorigenesis is supported by many in vitro and in vivo studies showing that increased expression of IL-13Rα2 promotes tumor progression in several tumor models including melanoma, head and neck cancer, triple negative breast cancer, pancreatic, ovarian, prostate cancer, and glioma. In most of these tumors, overexpression of IL-13Rα2 is well documented and is generally associated with bad prognosis. IL-13Rα2 is selectively overexpressed in approximately 60% of GB.[32] In particular, IL-13Rα2 has been shown to increase as the malignancy grade increases in gliomas, correlating with poor prognosis for patients.[27] It has also been shown to promote tumor invasion and migration, as well as to protect tumor cells from induction of apoptosis. Importantly, IL-13Rα2 appears to be expressed on cancer stem cells. It may be considered as a driver oncogene. Interestingly, IL-13Rα2 is not or very low expressed in normal tissues (except in testis, which is an immune-protected organ). Thus, IL-13Rα2 could be considered as a specific tumor-associated protein and a good target candidate for immunotherapy using a vaccine approach.

1.2.1.1.2. EO2317

EO2317 is a [redacted] microbiotal peptide with homology to survivin. Survivin is a member of the inhibitor of apoptosis gene family, which encodes negative regulatory proteins that function as endogenous inhibitors of caspases and preventing apoptotic cell death.[33] Survivin also has a role as a mitosis regulator, physically associated with the mitotic apparatus thereby ensuring the proper completion of various stages of cell division probably via the regulation of microtubule dynamics and stability.[34] These molecular functions are supported by a number of in vitro and in vivo preclinical studies that demonstrated that modulation of survivin expression reduced tumor growth, increased apoptosis, and sensitized tumor cells to chemotherapeutic drugs.[35] Overexpression of survivin in cancer may overcome an apoptotic checkpoint and favor aberrant progression of transformed cells through mitosis. While survivin is strongly expressed

in embryonic tissues where it plays a role in development, expression of survivin in normal adult tissue is scarce. Inversely, expression of survivin is observed in almost all type of neoplasms, including lung, colon, breast, pancreas, stomach, liver, ovary and prostate cancer, melanoma, hematopoietic malignancies, and glioma.[36] In glioma, expression of survivin has been shown to increase with malignancy grade with overexpression reaching 85% of Grade IV glioma (GB).[37] Survivin expression is associated with poor survival, older age, and higher WHO grade and is considered as a useful prognostic and diagnostic biomarker.[29] Overall, the large pattern of overexpression of survivin in tumor, associated with its critical role in tumorigenesis, made survivin an effective chemotherapy target[38] and immunotherapy target.[39] Furthermore, detection of survivin specific T cells in peripheral blood from glioma-affected patients further supports survivin as a viable target for vaccine approach.[40]

1.2.1.1.3. EO2318

EO2318 is a [redacted] peptide identified from human gut microbiome that targets FOXM1. It follows the same principles as for EO2316 and EO2317 so that it is highly homologous with the FOXM1 antigen overexpressed on tumor tissue. Forkhead box M1 is a member of the Fox transcription factors involved in G1-S and G2-M progression. FOXM1 exhibits a proliferation-specific expression pattern and its expression is regulated by proliferation and antiproliferation signals as well as by proto-oncoproteins and tumor suppressors.[41] While its expression is turned off in terminally differentiated cells, it is upregulated in a multitude of human solid tumors including breast cancer, non-small cell lung carcinoma, hepatocellular carcinoma, pancreatic carcinoma, colon cancer prostate cancer, and GB. Forkhead box M1 overexpression was a common molecular alteration in malignant glioma and the level of FOXM1 protein expression in human glioma tissues was directly correlated with the glioma grade.[28] As a transcription factor, FOXM1 has been shown to drive overexpression of number of oncogenes in glioma as SOX2 [42], vascular endothelial growth factor (VEGF) [43], and to confer resistance to temozolomide[44] and radiotherapy.[45]

1.2.1.1.4. UCP2

In order to generate an efficient immune response, the helper peptide UCP2 will be included in EO2401. This Th1 helper peptide will be able to sustain efficient dendritic cell activation and specific cytotoxic T cell activation.[46] UCP2 was initially described by Godet et al.[47] It is a telomerase-derived CD4+ epitope that binds to most commonly found human MHC class II alleles. The role of telomerase in tumor progression is well documented. Cancer cells overcome senescence via telomere length maintenance mechanisms involving telomerase activation.[48] Telomerase reverse transcriptase (TERT) overexpression achieved via multiple genetic and epigenetic mechanisms has been observed in 80% to 90% of malignant tumors.[49],[50] The use of a helper antigen targeting TERT in glioma is supported by the fact that mutations in TERT promoter are common in GBs, leading to enhance TERT transcription. TERT gene is proposed to be a prognostic and predictive biomarker of glioma and 75% of gliomas have enhanced telomerase activity.[51],[52] Ability of UCP2 to elicit specific CD4+ T cell responses have been demonstrated in in vivo models. Furthermore, spontaneous T cell responses against UCP2 were observed in various types of cancers,[53] and this peptide is currently under clinical evaluation in new small cell lung cancer (NCT02818426). All of these data strongly support the use of UCP2 as a helper peptide in EO2401.

[redacted]

[redacted]

1.2.1.2. [redacted]

[redacted]

1.2.1.3. Check Point Inhibitor in the Treatment of Malignant Gliomas

Check Point Inhibitors, approved for the treatment of different types of cancer, release the breaks on the immune system; however, only a small subset of patients treated with CPIs will achieve a clinical complete response (CR). Nevertheless, deep, durable, and rapid response on some tumor types makes these therapies promising to treat cancer.

The CPIs allow patient's existing T cell population to attack the tumor and, in order for this therapy to be effective, the patients must have corresponding T cells able to recognize the specific tumor antigens. It is likely that patients who do not partially respond to this therapy do not have sufficient numbers of T cells to overcome tumor growth. Therapeutic vaccination stimulates the immune system leading to the activation of the required T cell population for a more effective efficacy of the treatment allowing the T cell population to infiltrate the tumor thanks to the CPI.

The CPIs have been shown to be a very attractive treatment in other cancers and may optimally be used in combination with other therapeutic strategies, including radiation, and/or immunotherapies or other immunotherapies in the treatment of GB.[56]

1.2.1.3.1. Nivolumab

Programmed death-1 (PD-1) has been identified as an immune checkpoint pathway that tumor cells may exploit to evade immune surveillance. Tumor cells may block immune responses via the PD-1 immune checkpoint pathway by expressing the dual PD-1 ligands PD(L)-1 and PD(L)-2.

PD(L)-1 and PD(L)-2 engage the PD-1 receptor on T cells in order to inactivate T cells, which may allow tumor cells to evade immune response.

Check Point Inhibitors have demonstrated safety and clinical activity in different solid and hematological tumor types including in clinical trials in recurrent GB patients.

Nivolumab is an anti-PD-1, which is a fully human monoclonal antibody (immunoglobulin G4) promoting antitumor immunity allowing the immune system to attack the tumor, blocking the interaction between PD-1 and its ligands (PD[L]-1 and PD[L]-2), and stop a negative regulator of T cell activation and response, thus allowing the immune system to attack the tumor.

In a retrospective study of nivolumab for patients with recurrent high-grade glioma, the treatment showed a manageable safety profile and it was observed, in only a subset of patients, disease stabilization in heavily pretreated recurrent high-grade glioma.[57]

A recently completed Phase III CheckMate-143 study was the first randomized clinical trial in recurrent GB with the PD-1 CPI nivolumab as monotherapy. Although it did not meet its primary endpoint of improved OS over bevacizumab monotherapy, nivolumab may still have a place in the successful treatment of this disease, potentially as combination therapy, as a small subset (8%) of patients in the CheckMate-143 study did respond to nivolumab, and with a much longer duration of response (DoR; 11 months) than seen with bevacizumab therapy (5.3 months).[58]

Nivolumab is approved for use for the treatment of multiple cancer types including melanoma, Hodgkin lymphoma, squamous cell cancer of the head and neck, urothelial, renal cell

carcinoma, non-small cell lung, malignant pleural mesothelioma, colorectal cancer, hepatocellular carcinoma and esophageal cancer. It is not currently approved for GB.[60, 61]

1.2.1.3.1.1. Expected Adverse Events Related to Nivolumab

The following summary is based on the EU Summary of Product Characteristics (SmPC).[59]

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

In addition, nivolumab is associated with immune–related adverse reactions. With appropriate medical therapy, immune-related adverse reactions resolved in most cases. Table 3 presents the percentage for immune-related adverse reactions who were permanently discontinued from treatment by dosing regimen. Additionally, for patients who experienced an event, Table 3 presents the percentage of patients who required high-dose corticosteroids (at least 40-mg daily prednisone equivalents) by dosing regimen.

Note: for the laboratory abnormalities observed with nivolumab monotherapy, please refer to the European SmPC or to the US Product Information (PI).[59,60]

Of the 2085 patients who were treated with nivolumab monotherapy 3 mg/kg every 2 weeks and evaluable for the presence of anti-nivolumab antibodies, 11% tested positive for treatment-emergent anti-nivolumab antibodies by an electrochemiluminescent assay and 0.7% had neutralizing antibodies against nivolumab.[61]

Generally, no overall differences in safety regarding nivolumab were reported between elderly (≥ 65 years) and younger patients (< 65 years). Of the 1359 patients randomized to single-agent nivolumab in CHECKMATE-017, CHECKMATE-057, CHECKMATE-066, CHECKMATE-025, and CHECKMATE-067, 39% were 65 years or older and 9% were 75 years or older. No overall differences in safety or effectiveness were reported between elderly patients and younger patients.[61]

In the nivolumab non-squamous non-small cell lung cancer study (CA209057), the safety profile in patients with baseline renal or hepatic impairment was comparable to that in the overall population. These results should be interpreted with caution due to the small sample size within the subgroups.[60]

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

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

1.2.1.3.2. Combination of the EO2401 with a Check Point Inhibitor

The combination of a peptide vaccine and CPIs to treat cancer appears a promising therapy that will be evaluated in this clinical study. Clinical studies using dendritic cell vaccines, peptide-based vaccines, and immune CPIs are underway. It is likely that combination regimens with complementary mechanism of action will ultimately be required to achieve a broad and durable tumor response.[61]

To demonstrate the antitumoral effect of vaccine and the synergic effect with CPIs, an adoptive transfer of T cells in nude mice engrafted with U-87MG cells was performed. Immunocompromised nude mice were injected SC into the flank region with U-87MG human

GB cells.[62] U-87MG cells are positive for IL-13Rα2 and HLA-A2 and also express PD(L)-1 which enables treatment of the tumor-bearing mice with an anti-PD-1 antibody (clone RMPI-14, BioXcell) in combination with the vaccination approach. After 21 days, the tumor size was determined and the animals were randomized in different groups for treatment with anti-PD-1 antibody and adoptive transfer of T cells. T cells for adoptive transfer were obtained from HHD DR3 immunized mice.[62] [redacted]

The study design is shown in Figure 2 and tumor growth curves in Figure 3.

[redacted]

[redacted]

[redacted]

Data are presented as median plus error for each group.

[redacted]

These results obtained in tumoral model strongly support the use of bacterial peptides for an antitumoral therapeutic vaccine in combination with anti-PD-1.

1.2.1.3.3. Bevacizumab

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.

Worldwide, bevacizumab is authorized in different settings including colorectal cancer, non-squamous non-small cell lung cancer, renal cancer, cervical cancer, and ovarian/fallopian tube cancers. In addition, in the United States, bevacizumab is authorized for treatment of adult patients with recurrent GB.

1.2.1.3.3.1. Expected Adverse Events Related to Bevacizumab

The following summary is based on the EU SmPC.[63]

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.

1.2.2. Nonclinical Studies

A series of nonclinical safety pharmacology and toxicity studies have been conducted with the EO2315 in the context of proof-of-concept studies, EO2316, EO2317, or EO2318 in mice to support the use of EO2401 in this FIH study. Findings of relevance to this protocol are provided below. Further details are provided in the Investigator's Brochure.[62]

1.2.2.1. Immunogenicity of EO2316, EO2317, and EO2318

Immunogenicity of candidate peptides and cross reactivity in [redacted]

EO2316 - [redacted]
immunization of mice with EO2316 allowed inducing T cells that were able to react strongly after challenge with either EO2316 or human peptide demonstrating cross reactivity. Thus,

EO2316 is strongly immunogenic and is able to drive an effective immune response against human peptide.

EO2317 and **EO2318** - [redacted] Immunization of mice with EO2317 or E02318 was followed by a high ex-vivo response of splenocytes against the same bacterial peptide, thus demonstrating the strong immunogenicity of EO2317 or EO2318. The cross reactivity of these cells induced by EO2317 or EO2318 against the human corresponding peptides was demonstrated.[62]

In summary, these immunogenicity studies showed that the 3 candidate peptides, EO2316, EO2317, and EO2318, induced stronger immune responses than the corresponding human peptides and were able to drive an effective immune response against the corresponding IL-13Rα2, BIRC5 (survivin), and FOXM1 human peptides. [redacted]

For further details, refer to the Investigator's Brochure.[62]

Immunogenicity of the helper peptide in [redacted]

[redacted]

[redacted]

[redacted] These results support the use of UCP2 as helper peptide in the intended clinical trial with EO2401.

[redacted]

Potential antitumor effect of the peptide candidates in a mouse xenograft GB model

An important nonclinical investigation, including a tumoral model, strongly supporting the use of bacterial peptides for a therapeutic vaccine in combination with anti-PD-1 has been presented in Section 1.2.1.3.2.

1.2.2.2. Toxicology Studies

Although HLA-A2 transgenic animals have been used to demonstrate that the individual peptides in EO2401 DP peptide mixture solution are immunogenic, [redacted]

So far, nonspecific adverse effects that may occur following binding of the peptides to HLA molecules have not been observed in HLA-A2 transgenic mice. In addition, clinical trials have been completed or are ongoing using IL-13Rα2, BIRC5 (survivin), or FOXM1 as a target for peptides vaccination (NCT01130077[64]; NCT02078648[65]). In all of these trials, there were no treatment limiting AEs associated with the targeting of IL-13Rα2, BIRC5 (survivin), and FOXM1.

The immune response and cross reactivity against mice peptide homologs were performed on standard C57BL6 and BALB/C mice and the additionally assessed safety parameters revealed no visible sign of toxicity:

- No body weight change
- No change in colon length nor weight
- No bleeding in the stools
- No signs of overt colitis
- All organs, including intestine, appeared macroscopically normal.

Universal cancer peptide 2 is used as a CD4+ helper peptide in some ongoing clinical trials, including in patients with non-small cell lung cancer (NCT02818426).

[redacted]

For further details, refer to the Investigator's Brochure.[62]

1.2.3. Clinical Studies & Rationale and High Level Outline for Global Amendment #2 (leading to protocol EOGBM1-18 version 3)

To date, no final human trial data is available on safety and efficacy of EO2401, as the early clinical development trials are still running.

EO2401 is evaluated in two phase 1/2 trials running in parallel, trial EOGBM1-18 in patients with recurrent glioblastoma (EudraCT#: 2018-002279-16; IND#: 19229), and trial EOADR1-19 in patients with adrenal tumors (adrenocortical carcinoma [ACC], and malignant pheochromocytoma/paraganglioma [MPP]) (EudraCT#: 019-003396-19; IND#: 19229); both trials are conducted under the supervision of Independent Data Monitoring Committees (IDMCs).

Trial EOGBM1-18, the current trial, is an international, multicenter, open label, multi-cohort study, recruiting patients at 10 sites in Germany, France, Spain, and USA, where the first patient started study treatment July 28, 2020. At an IDMC meeting [redacted], there was a consensus decision, after 3 evaluable patients had been treated without any reported safety concern event (i.e. no related adverse event of grade 3, or higher, had been reported), to recommend finalizing the recruitment to Cohort 1 (safety lead-in, 3-by-3 designed cohort) of trial EOGBM1-18, and to open Cohorts 2a and 2b for recruitment. Based on a further 3 evaluable patients (without any safety concern events) treated in Cohorts 2a/2b, the IDMC [redacted] arrived at a consensus decision to recommend also opening of Cohort 3 of the trial.

[redacted]

[redacted]

The second early clinical development trial including EO2401, i.e. EOADR1-19, is an international, multicenter, open label, multi-cohort study, recruiting patients at 10 sites in France, Germany, Italy, Netherlands, Spain, Denmark, Sweden, and USA. The study population is male and female HLA-A2 positive patients, at least 18 years old with either previously untreated, or previously treated, ACC or MPP. Cohort 1 of the trial (first patient starting study treatment August 11, 2020), assumed to be most important from a general safety perspective, included previously treated patients with ACC and MPP, and an evaluation by a 3-by-3 design of EO2401 in combination with nivolumab. At an IDMC meeting [redacted] there was a consensus decision, after 3 evaluable patients had been treated without any reported safety concern event (i.e. no related adverse event of grade 3, or higher, had been reported), to recommend finalizing the recruitment to Cohort 1 of trial EOADR1-19, and to open Cohorts 2A and 3A for recruitment (patients with previously treated disease). Based on a further 3 evaluable patients (without any safety concern events) treated in Cohorts 2A/3A, the IDMC at a meeting [redacted] arrived at a consensus decision to recommend also opening of Cohorts 2B and 3B (patients with no previous treatment for advanced/metastatic disease).

The IDMCs of trials EOGBM1-18 and EOADR1-19 receives monthly safety updates from the Sponsor, and there has not been any need to call for any further IDMC meetings beside the pre-planned meetings outlined above [redacted] since there has been no safety concern event (as defined by the trial protocols and IDMC Charters) reported in any of the trials. The safety lead-in cohorts in both trials run with minimum number of patients (each 3 patients) due to lack of safety issues (no safety concern events detected), and all phase 2 cohorts in both trials have been opened as planned for recruitment.

The continuously ongoing safety assessment by the Sponsor regarding trials including EO2401 is showing that the major added event type to the background treatments (i.e. nivolumab and nivolumab/bevacizumab, depending on trial and cohort) are the expected events of mild local administration site skin reactions (e.g. erythema, induration, and pain). [redacted]

[redacted]

[redacted]

- [redacted]

- [redacted]

[redacted]

[redacted]

[redacted]

The above findings from the early part of trial EOGBM1-18, including 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 the rationale for a second global protocol amendment (leading to protocol EOGBM1-18 version 3) to expand the trial to further optimize the treatment schedule and

patient management to achieve an as long study treatment duration as possible to increase the possibility to achieve enhanced efficacy, and to investigate the feasibility to administer study treatment in the neoadjuvant setting.

The amendment is included in detail in the following protocol sections, especially **Section 3.1 Overall Study Design and Plan Description**, and **Section 6.3 Permitted Concomitant Medications**, and summarized at a high level in the following:

- *Reminder regarding EOGBM1-18 Version 2 treatment schedules: the only difference between Cohort 1a and Cohort 2a (and Cohort 2b, which is the same as Cohort 2a) is that in Cohort 1a the two first study drug administrations are given as EO2401 monotherapy two weeks apart (for Cohort 2a nivolumab is administered together with EO2401 from the start of treatment), then after a further 2 weeks the two regiments are the same and EO2401 is administered together with nivolumab. Cohort 3, only open for recruitment in the USA, include a combination treatment of EO2401/nivolumab/bevacizumab (bevacizumab dose 10mg/kg every 2 weeks according to the FDA label); currently 8 patients are in treatment with this regimen (from 1 to 12 weeks, and ongoing) and no safety concern events have been reported.*

- Addition of 12 patients to Cohort 1a (i.e. same treatment schedule as Cohort 1a in the safety lead-in part of the trial, but with the safety measures as for Cohort 2a; also same inclusion/exclusion criteria, i.e. measurable disease, as for the initial safety lead-in cohort and Cohort 2a), and inclusion of extended patient management measures (see below); rationale is that only 3 patients have been treated with delayed nivolumab treatment and all of the 3 patients have comparably long treatment durations (i.e. a possible efficacy signal which needs to be substantiated).

- Addition of 10 patients to Cohort 2a without any change of the schedule or inclusion criteria (i.e. measurable disease), and inclusion of extend patient management measures (see below); rationale is that the schedule with combination of EO2401/nivolumab from start seems to give a faster and more extensive T cell expansion (preferred pattern) than the delayed nivolumab schedule, but without adjusted patient management measures comparable short treatment durations; thus, goal is to, if possible, prolong treatment duration by more aggressive patient management at situations possibly being symptomatic PsPD.

- Addition of 6 patients in a new Cohort 2c for neoadjuvant study treatment (EO2401plus nivolumab administered twice with 2 weeks interval; thus, population include patients scheduled for clinically indicated resection of first recurrent glioblastoma), confirmed by an interim MRI at day 14 (i.e. at V2), followed by planned surgery, and thereafter adjuvant study treatment (EO2401 plus nivolumab administered twice with 2 weeks interval, then switch to EO2401 on a 4-weekly schedule and continued nivolumab 2-weekly), including also extended patient management measures (see below). The rationale for the new cohort is to preliminary assess safety and feasibility of a neoadjuvant/adjuvant treatment strategy, which already have shown some promise utilizing anti-PD1 blockade only.[100, 101] In addition, the treatment setting allows assessments of immuno-biological effects in tissue of the combined treatment of EO2401 and anti-PD1 blockade.

- Inclusion of immune testing in all running cohorts also at the visit after the first EO2401 administration (V2); rationale is to investigate how early an expansion of T cells occur

(in EOGBM1-18 version 2, on study testing started at V3) to further advise on possible schedule optimizations (note, to keep blood sampling constant, testing at a later timepoint is cancelled in patients where V2-testing is implemented).

- Addition of extended patient management in all running cohorts to ameliorate significant neurological symptoms; rationale is to avoid early study treatment stopping and at least allowing as many patients as possible to get to a confirmation of progression according to iRANO criteria, or the possibility detecting that the symptomatic progression could be related to PsPD. Structures measures to be included are:

    - In situations where added supportive medication is judged as medically appropriate by the treating physician due to patient brain edema with symptoms, low-dose bevacizumab for up to three months unless medically contra-indicated can be administered. The number of bevacizumab doses to be given during the three months window will be determined by the treating physician based on her/his assessment of the best interest of the patient. When bevacizumab is administered for symptomatic cerebral edema, study therapy may be continued at the discretion of the treating physician.

        - The rationale to use low-dose bevacizumab for treatment of brain tumor edema, for instance in relation to radiotherapy induced necrosis, is well established, and the literature has been summarized.[99] An important trial to substantiate the use of low-dose bevacizumab was published by Levin et al 2011[102], providing class I evidence (randomized double-blind placebo-controlled trial) of bevacizumab efficacy in the treatment of CNS radiation necrosis justifying consideration of this treatment option for people who suffer radiation necrosis secondary to the treatment of head and neck and brain cancers. Importantly, the pathophysiology behind radiation therapy induced necrosis, i.e. damage resulting from local cytokine release, increase in capillary permeability, and extracellular edema, is the pathophysiology at hand also in a situation of intense immune cell attack of a brain tumor.

    - In situations where symptomatic cerebral edema is more significant or anticipated to evolve to become more significant, the treating physician may also prescribe a high dose of dexamethasone followed by a rapid taper as tolerated and as clinically indicated. During a dexamethasone high dose regimen, study therapy should be interrupted.

[redacted]

- [redacted]

- [redacted]

[redacted]

- [redacted]

- [redacted]

[redacted]

- [redacted]

- [redacted]

[redacted]

- [redacted]

- [redacted]

- [redacted]

In addition to above, two further types of changes are included in global amendment 2 (leading to EOGBM1-18 version 3):

- Recommendations regarding vaccination against SARS-CoV-2/COVID-19 as applied in trial EOGBM1-18 since January 2021 and forward via investigator communications, are included in **Section 6.3**.

- Updates of management principles for immune-related adverse events as outlined in **Section 7.6.1.1 and Table 5**, based on the most recently available labelling documents for Opdivo (SmPC August 3, 2021[59]) and standard protocol guidance from Bristol-Myers Squibb (market authorization holder for nivolumab). In light of these adjustments, and to also follow standard protocol guidance from Bristol-Myers Squibb and align protocol EOGBM1-18 with protocol EOADR1-19 exclusion criteria regarding laboratory value exclusions have also been updated.

1.2.4. Cohort 3 clinical outcomes & Rationale and High-Level Outline for Global Amendment #3 (leading to protocol EOGBM1-18 version 4)

Safety of EO2401/nivolumab +/- bevacizumab

As outlined in **Section 1.2.3**, IDMC assessments of the safety lead-in parts of both trials currently investigating EO2401 (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.

From a safety perspective [redacted]

An overall assessment of safety, at this stage of study conduct, does not show any clear differences between patients treated with EO2401/nivolumab and EO2401/nivolumab/bevacizumab, and in this context the combined safety profile of all patients treated in trial EOGBM1-18 will be referred to below.

[redacted]

[redacted]

[redacted]

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

[redacted]

[redacted]

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

[redacted]

Safety conclusion

In conclusion, the combination of EO2401 [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.

Of note, administration site reactions seen in trial EOGBM1-18 are below, or well within the expected frequency for any antigen [redacted]

[redacted]

[redacted]

Global amendment 3 leading to protocol EOGBM1-18 version 4

At a high level the amendment includes the following items and rationale:

- Expansion of Cohort 3 and broadening of recruitment outside of the USA:

  Cohort 3 include a combination treatment of EO2401/nivolumab/bevacizumab (bevacizumab dose 10mg/kg every 2 weeks according to the FDA label) and was only open for recruitment in the USA during conduct of protocol versions 1-3.

  [redacted]

  The intention is also to open the trial for recruitment at sites outside of the USA for Cohort 3 to broaden the base of sites with experience of the regimen. The broadening of the recruitment base is not assumed to influence the safety profile of the treatment since bevacizumab, even if not labelled in large parts of Europe, is a not seldom used compound for treatment of recurrent glioblastoma also in Europe.

  A further reason to expand Cohort 3 is also to enhance the possibility to compare the continuous use of standard dose bevacizumab (as in Cohort 3) with the neurological

symptom directed use of low-dose bevacizumab as instituted in protocol version 2 (mainly used in Cohorts 1a and 2a).

- [redacted]

1.3. Benefit-risk Assessment

Glioblastoma is the most lethal brain tumor with 25% of patients surviving up to 2 years and less than 5% of patients making it to a 5-year post-diagnosis milestone.[18,19] As of today, few effective treatment options exist against progressive and recurrent GB, which occurs in most patients following surgery and combined radiotherapy/temozolomide treatment delivered as primary therapy.[18,19]

Repeat resection is the best available method for prolonging the survival of selected patients with recurrent GB, especially those with a mass effect. However, prognostic factors, such as age, performance status, and presumed maximal extent of resection, which must always be considered, may indicate that repeat resection may not be the appropriate therapeutic option.

As with repeated resection, a lack of prospective randomized trials, and bias in selecting patients for single-arm trials, prevent any reliable conclusions from being made regarding the benefit provided by repeated irradiation in patients with recurrent malignant glioma.

According to treatment guidelines, systemic chemotherapy is an option for treatment of patients with recurrent GB; even though outcomes are not impressive, clinical trials might be preferred for eligible patients.[66,67] Lomustine is one possible chemotherapy treatment at recurrence and a recent trial where this compound was administered as monotherapy (n = 60) indicates the following efficacy: median OS 5.6 months, median PFS 1.9 months, and objective response rate (ORR) of 3%.[68] Targeted treatments await their full breakthrough and utilization in GB, even though bevacizumab (anti-VEGF monoclonal antibody) has already achieved an authorization for treatment of adult patients with recurrent GB in the United States.[69]

Bevacizumab has shown marked radiographic responses and improvement in PFS versus controls, but Phase III studies have failed to demonstrate an OS advantage.[70]

The CheckMate-143 clinical trial was the first large-scale randomized clinical trial of 2 targeted therapies in recurrent GB.[71] Patients received either nivolumab (anti-PD-1 monoclonal antibody) 3 mg/kg or bevacizumab 10 mg/kg IV every 2 weeks, until disease progression or unacceptable toxicity. At the time of final analysis, this Phase III clinical trial enrolled 369 patients with first recurrence of GB, previously treated with combination radiation and temozolomide. The trial failed showing an extension of OS by nivolumab treatment as compared to bevacizumab. In both treatment groups, the 12-month survival was 42%. The median survival for patients treated with nivolumab was 9.8 months as compared to 10 months for those receiving bevacizumab monotherapy. Median PFS was 1.5 months (nivolumab) and 3.5 months (bevacizumab). Overall treatment response rates were 8% (nivolumab) and 23% (bevacizumab), and median durations of response were 11.1 months (nivolumab) and 5.3 months (bevacizumab).

Thus, the targeted therapies nivolumab and bevacizumab have both shown some level of activity in GB. However, it seems as the efficacy of the compounds as monotherapies might not be potent enough, but paving the way for therapeutic options adding further rational combination partner to enhance activity and prospects for patient with recurrent GB.

To date, no final human trial data is available on safety and efficacy of EO2401 in combination with nivolumab, as the early clinical development trials are still running. However, safety data is available from two trials, the current trial EOGBM1-18 and a trial including patients with adrenal tumors (EOADR1-19), showing a safety profile currently indicating that the major added event type by EO2401 to the background treatments (i.e. nivolumab and nivolumab/bevacizumab, depending on trial and cohort) are the expected events of mild local administration site skin reactions (e.g. erythema, induration, and pain); see further **Section 1.2.3**.

The immunogenicity results obtained with EO2316, EO2317, and EO2318 main part of EO2401 in mouse models and the antitumor effect of the EO2315 peptide – used in proof-of-concept studies – when combined with anti-PD-1 in a mouse xenograft GB model strongly support the use of the EO2401 in combination with anti-PD-1 in the treatment of GB.

The combination of EO2401 and nivolumab is intended to increase the EO2401-specific T cell response infiltration in the tumor and the killing effects directed against the tumor cells. This initial response might also lead to antigen spreading through the killing of the tumor cells induced by EO2401 and the induction of responses to other antigens than the ones of EO2401. Clinical trials were conducted to evaluate the safety and the efficacy of a vaccine and a CPI combination therapy of a vaccine and a CPI. The first results showed an acceptable safety profile comparable to the safety profile of the CPI alone. Consequently, the safety profile of EO2401 in combination with nivolumab was before the start of trials EOGBM1-18 and EOADR1-19 expected to be very close to the safety profile observed with the nivolumab as monotherapy.[54] An expectation which based on early data seems to be moving towards being validated (see **Section 1.2.3**).

The addition of bevacizumab to the EO2401 and nivolumab combination is supported by the fact that GB 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.[72]

Bevacizumab blockade of VEGF is thus assumed to enhance the possibilities for EO2401 and nivolumab to work. In addition, bevacizumab gives the possibility for patients to possibly have a slightly prolonged time to progression (even if survival is not prolonged) meaning that patients might benefit from a longer time administration of EO2401 plus nivolumab.

Anti-PD-1 blockade in combination with bevacizumab has been evaluated in combination in patients with recurrent GB and found to be well tolerated.[73,74] Before the Cohort 3 (including EO2401, nivolumab, and bevacizumab) in the current trial will be opened (after a recommendation from the Independent Data Monitoring Committee [IDMC]), the safety and tolerability of the combination of EO2401 in combination with nivolumab has been assessed in Cohorts 1 and 2.

From a risk perspective, based on the above and already achieved clinical experiences utilizing efficacious immunization approaches together with anti-PD-1 blockade[54], the assessment of the Sponsor is that the safety profile of the combination of EO2401 and nivolumab will be similar to the well described safety profile of nivolumab, to which no addition of serious additional non-specific off-target safety issues are expected. Likewise, the assessment of the Sponsor is that the safety profile of the combination of EO2401 and nivolumab/bevacizumab will be similar to the safety profiles of the combination compounds.

However, provided EO2401 induces an efficacious expansion of T cells specific for the targeted TAAs with ability to kill tumor cells expressing one, or multiple, of these TAAs, it cannot be ruled out that such antigen specific T cells might also recognize these antigens if expressed, and presented by MHC, at a high enough level on non-cancerous cells. Thus, if killing of tumor cells by T cells expanded by EO2401 immunization could occur, normal cells might also be killed by such T cells leading to symptoms like the immune-related adverse reactions which might be seen during treatment with anti-PD-1 (or similar compounds) alone (e.g. see the European SmPC[59] or the US PI[60] for nivolumab). 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 EO2401 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 7.6.1.1**).

In addition to above, as for all immunization approaches some degree of mild to moderate local injection events of short duration might be expected for EO2401. Also, as for all administrations of immunogens, it cannot be excluded that immediate type allergic reactions might occur at injections of EO2401; special precautions are outlined for this type of potential reactions (**Appendix 7 Infusion/Treatment related Reactions**).

The following study procedures will ensure safety monitoring along the clinical trial:

- The clinical team at investigational sites (which are expert centers) will be made aware of the potential side effects in order to manage the side effects efficiently
- The patients will be monitored closely during the treatment period through regular and frequent visits including clinical, laboratory, and radiological exams
- 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 trial conduct. [redacted] and the IDMC will recommend on possible expansions in Cohort 1, and transition from Cohort 1 to 2a and 2b, and from Cohorts 2a and 2b to 3.

Given the potential for therapeutic benefit of EO2401 combined with nivolumab, and nivolumab/bevacizumab in patients with progressive GB based on published data (including some inborn activity of both nivolumab and bevacizumab as individual therapies in GB; see above), the promising tumor model data (showing the synergy of the microbiome-derived peptide and anti-PD-1) and the nonclinical toxicology data available at present support the start of this Phase Ib/IIa trial.

The findings from the early part of trial EOGBM1-18 described in **Section 1.2.3 and Section 1.2.4**, including 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 the rationale for a third global protocol amendment and supports the positive benefit-risk for expansion of trial EOGBM1-18 for treatment of further patients according to the third global amendment (leading to EOGBM1-18 version 4).

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objective

The primary objective of this FIH trial of EO2401 is to evaluate the safety and tolerability of EO2401 as monotherapy and in combination with nivolumab, and nivolumab/bevacizumab in patients with progressive GB. The safety and tolerability evaluation include assessments of different patient populations within the group of patients with recurrent GB: i) Cohorts 1a, 2a, and 3 evaluate patients with measurable disease, ii) Cohort 2b evaluates patients with non-measurable disease (after surgery of recurrent disease, i.e. adjuvant treatment), and iii) Cohort 2c evaluates a neoadjuvant/surgery/adjuvant treatment concept.

2.1.2. Secondary Objectives

The secondary objectives are:

- The evaluation of survival
- The evaluation of tumor progression and response by MRI assessment using the Immunotherapy Response Assessment in Neuro-Oncology (iRANO) criteria
- To assess the immunogenicity of EO2316, EO2317, EO2318, and UCP2 that compose EO2401 by IFN-γ ELISpot, and by intracellular cytokines staining, or multimers staining assays. Cross reactivities with the human TAAs IL-13Rα2, BIRC5 (survivin), and FOXM1 will also be tested.

2.1.3. Exploratory Objectives

- To assess the neurologic function assessed by the Neurologic Assessment in Neuro-Oncology (NANO) scale (see Appendix 2[75,76])
- To explore the correlation between immunogenicity of EO2316, EO2317, EO2318, and UCP2 that compose the EO2401 and tumor progression and response outcome parameters
- [redacted]
- To assess humoral immune responses toward EO2316, EO2317, EO2318, and UCP2 that compose the EO2401 [redacted]
- To assess possible T cell expansions due to antigen spreading [redacted]
- [redacted]

[redacted]

2.2. Endpoints

2.2.1. Primary Endpoint

The primary endpoint includes the incidences of AEs, treatment-emergent AEs (TEAEs), SAEs, deaths, and laboratory abnormalities using the National Cancer Institute-Common Terminology Criteria for AEs (NCI-CTCAE) v5.0.

Safety endpoints will be assessed by review of summaries of AEs/TEAEs/SAEs, unless otherwise stated. Adverse events will be categorized by system organ class (SOC) and preferred term (PT) using the current Medical Dictionary for Regulatory Activities (MedDRA) version and will be graded according to NCI-CTCAE v5.0.

2.2.2. Secondary Endpoints

The secondary endpoints are:

- Overall survival, defined as the time interval from the date of first study treatment administration to the date of death due to any cause
- The tumor progression and response by MRI measurements every 8 weeks using the iRANO criteria evaluated by:
    - Progression-free Survival, using the iRANO criteria (see Appendix 1[77,78]) and defined as the time interval from the date of first study treatment administration to the date of first occurrence of progression or death from any cause, whichever occurs first
    - Objective Response Rate (ORR), defined as the proportion of patients with complete response (CR) and partial response (PR) according to the iRANO criteria
    - Disease Control Rate (DCR), defined as the proportion of patients with CR, PR, and Stable Disease (SD) according to the iRANO criteria
    - Duration of Response (DoR), defined as the time interval from the date of first occurrence of CR or PR to the date of first documentation of disease progression or death from any cause, whichever occurs first.
- The immunogenicity of EO2316, EO2317, EO2318, and UCP2 that compose EO2401 by [redacted]

[redacted]

2.2.3. Exploratory Endpoints

The exploratory endpoints will be:

- Neurologic function assessed by the NANO scale (see Appendix 2[75,76])
- Correlation between immunogenicity of EO2316, EO2317, EO2318, and UCP2 that compose EO2401 and the outcome parameters [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

3. INVESTIGATION PLAN

3.1. Overall Study Design and Plan Description

This is a multicenter, Phase Ib/IIa, FIH study to assess the safety, tolerability, immunogenicity, and preliminary efficacy of EO2401 in patients with unequivocal evidence of progressive or first recurrent GB confirmed by MRI as defined by the Response Assessment in Neuro-Oncology (RANO) criteria (note: within the first 3 months after completion of radiotherapy and concomitant temozolomide, diagnosis of recurrence can be indistinguishable from pseudoprogression [PsPD] on neuroimaging).[66]

The FIH protocol is an open-label, multi-cohort study intended to recruit a maximum of approximately 100 evaluable patients in total:

- Cohort 1 (as study safety lead-in, before global amendment 2) includes an evaluation by a 3-by-3 design of EO2401 monotherapy (2 administrations of EO2401 each followed by a 2-weeks observation period) planned to be followed in the same individual patients by continued EO2401 in combination with nivolumab; 3 to 12 evaluable patients will be included depending on the safety profile of the administered treatments
    - In the safety lead-in part (3-by-3 design) of the trial, 3 evaluable patients started treatment in Cohort 1a, after which the safety lead-in was closed after a recommendation from the IDMC, and Cohorts 2a and 2b opened
    - In global amendment 2 (EOGBM1-18 version 3), plus via re-distribution of patients from Cohort 2b, 18 evaluable patients were added to Cohort 1a for further evaluation of the treatment schedule with a 4-weeks delayed start of nivolumab (safety precautions for the added patients will follow the same principles as for Cohorts 2a, 2b, 2c, and 3, since Cohort 1a is no longer a safety lead-in part of the trial) (recruitment finalized before global amendment 3)
- Cohorts 2a and 2b are an evaluation of EO2401 in combination with nivolumab (both compounds started at the same time) planned to include approximately 48 evaluable patients in total (patients in Cohort 2a with at least one measurable lesion and patients in Cohort 2b with no measurable enhancing disease).
    - Before global amendment 2 (EOGBM1-18 version 3), 23 evaluable patients started treatment in Cohort 2a (after re-distribution of patients between Cohort 1 and Cohort 2a to keep a relevant safety database; see further **Section 1.2.3**)
    - In global amendment 2 (EOGBM1-18 version 3), plus via re-distribution of patients from Cohort 2b, 15 evaluable patients were added to Cohort 2a for further evaluation of the treatment schedule with simultaneous start of EO2401 and nivolumab, but with inclusion of extended patient management measures (recruitment finalized before global amendment 3)
    - Cohort 2b was planned to include 15 evaluable patients both before and after global amendment 2 (EOGBM1-18 version 3), but number of patients lowered to 6 by re-distribution of patients to Cohorts 1a and 2a (recruitment finalized before global amendment 3)
- Cohort 2c is a cohort introduced by global amendment 2 (EOGBM1-18 version 3), to include 6 evaluable patients to assess safety and feasibility of a neoadjuvant/adjuvant

treatment strategy including EO2401/nivolumab (both compounds started at the same time)

- Cohort 3 is an evaluation of EO2401 in combination with nivolumab and bevacizumab planned to include 10 evaluable patients before global amendment 3 which increased the target recruitment to 26 patients. [redacted]

Generally, patients evaluable for safety will include all patients who have received at least 1 dose of EO2401. [redacted] Patients evaluable for efficacy based on the Per-Protocol (PP) population will consist of any patients who received at least 3 [redacted] for whom no important protocol deviations occurred and have at least 1 evaluable post-Screening tumor assessment (tumor assessment is not necessary if a clinical/pathologically defined progressive disease [PD] is defined). Patients who are not considered evaluable for the PP population will not be replaced, and be included in the Full Analysis Set (FAS).

Eligible patients who meet the inclusion criteria and do not meet any of the exclusion criteria and have provided informed consent will be enrolled in the study in Europe and/or the United States. [redacted]

The study treatments will be administered until confirmed tumor progression, intolerable toxicity, death, Investigator or patient decision, or early termination of the study at the request from the Sponsor. At the time of stopping study treatment, appropriate standard of care will be initiated by the Investigator. The patient should continue study follow-up measures 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 by the Sponsor.

For all cohorts, and all patients during the whole trial treatment, the following stopping safety rules will apply:

- [redacted]

- [redacted]

[redacted]

- The only adjustments regarding dose for the EO2401 combination partners nivolumab and bevacizumab 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[59,63] or the US PI[60,69], which are available for both compounds. Dose adjustments for EO2401 also include the potential of withholding one or several doses (see **Section 7.6.1.1**), or discontinuation of treatment. In addition, a lowered dose might be applied in the potential transition from sub-cohort 1b to sub-cohort 1c in relation to safety events (see further below)
- In instances where the labels for nivolumab and/or bevacizumab prescribe that the drug 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 EO2401 alone or EO2401 in combination with nivolumab or bevacizumab, respectively) provided such a continuation would be recommended by the IDMC and supported by the Sponsor. If EO2401 would be stopped, the other drugs in the combination will also be stopped in

the context of being trial treatment. In such cases, the Investigator responsible for the patient in question will advise on adequate further treatment options

- In case of infusion-related reactions and hypersensitivity reactions, please refer to Appendix 7.

Details of the IDMC process and procedures, and cohort management plan are outlined in a separate IDMC Charter.

[redacted]

Cohort 1 (see Figure 5)

Cohort 1 in the safety lead-in part of the trial will be evaluated in a 3-by-3 design to assess, first safety and tolerability of EO2401 alone during a 4 weeks period (2 administrations of EO2401, each followed by a 2-week observation period), planned to be followed in each patient by further administrations of EO2401 in combination with nivolumab for assessment of safety, tolerability, immunogenicity, and preliminary efficacy of the combination. The safety lead-in part of the trial included 3 evaluable patients starting treatment in Cohort 1a, after which the safety lead-in was closed after a recommendation from the IDMC, and Cohorts 2a and 2b opened.

In global amendment 2 (EOGBM1-18 version 3), plus via re-distribution of patients from Cohort 2b, 18 evaluable patients were added to Cohort 1a for further evaluation of the treatment schedule with a 4-weeks delayed start of nivolumab, i.e. with the same schedule as for Cohort 1 described in this section, but with extended patient management measures (see **Section 6.3**). Recruitment to Cohort 1 was finalized before global amendment 3.

Priming injections of EO2401 will be started as soon as possible after confirmation of disease progression; study baseline/screening MRI should be done no longer than 14 days before the start of treatment (MRI confirming progression of GB might be done earlier). The initial dosing regimen will be 4 priming injections administered SC at 2-weekly intervals for the first 6 weeks, followed by monthly boosting injections of EO2401 starting at 4 weeks after the fourth priming injection (i.e., at Week 10). In sub-cohorts 1a and 1b, the full dose of EO2401 (i.e., 1 mL of emulsified EO2401 DP in adjuvant) will be administered, and if implemented the sub-cohorts 1c and 1d will include half the dose of EO2401 (i.e., 0.5 mL of the emulsion will be injected). Thus, Cohort 1a after global amendment 2 (EOGBM1-18 version 3) should also include full dose of EO2401 (i.e., 1 mL of emulsified EO2401 DP in adjuvant).

Nivolumab will be administered as an IV infusion at a dose of 3 mg/kg every 2 weeks from the third priming injection of EO2401 (i.e., from start of Week 4). The nivolumab infusion is to start [redacted] after the EO2401 administration.

The safety precautions for Cohort 1a after global amendment 2 (EOGBM1-18 version 3) will follow the same principles as for Cohorts 2a, 2b, 2c, and 3, since Cohort 1a is no longer a safety lead-in part of the trial. Thus, for the added patients to Cohort 1a the general safety rules as described above applies, i.e. at related clinical Grade 4/5 AEs the study treatment in the individual patient experiencing the event will be stopped for Grade 4 AEs and 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.

In Cohort 1, as study safety lead-in, before global amendment 2 (i.e. these rules are NOT applicable to patients added to Cohort 1a by global amendment 2), the patients' recruitment was conducted in accordance to the following rules:

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- **Sub-cohort 1a (full dose of EO2401) 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 4 weeks in the individual patient, the IDMC will assess the totality of available safety data and recommend for decision by the Sponsor for the further recruitment steps (assumed would be opening recruitment of Cohorts 2a and 2b if there are no general safety concerns)
  - [redacted]
  - [redacted]
  - [redacted]

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 EO2401 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 EO2401), 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 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 2a and 2b if there are no general safety concerns)
  - [redacted]
  - [redacted]
  - [redacted]

[redacted]

- [redacted]

- **Sub-cohort 1c (half dose of EO2401), 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 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 Cohorts 2a and 2b if there are no general safety concerns; [redacted]
  - [redacted]

- [redacted]

  - [redacted]

[redacted]

- **Sub-cohort 1d (half dose of EO2401), 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 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 Cohorts 2a and 2b if there are no general safety concerns; [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Cohorts 2a and 2b (see Figure 6A)

Cohorts 2a and 2b are 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 Cohorts 2a and 2b will include administration of EO2401 in combination with nivolumab from the start of treatment in the individual patients for assessment of safety, tolerability, immunogenicity, and preliminary efficacy of the combination. Cohorts 2a and 2b were planned to include a total of approximately 48 evaluable patients, but was with amendments and re-distribution of patients finally including 44 patients divided as follows:

- Cohort 2a before global amendment 2, 23 evaluable patients
- Cohort 2a after global amendment 2, plus via re-distribution of patients from Cohort 2b, 38 evaluable patients (addition of 15 evaluable patients)
- Cohort 2b, planned 15 patients (both before and after global amendment 2), but number of patients lowered to 6 by re-distribution of patients to Cohorts 1a and 2a
- Recruitment to Cohorts 2a and 2b finalized before global amendment 3

Patients in Cohort 2a with at least one measurable lesion and patients in Cohort 2b with no measurable enhancing disease (i.e. Cohort 2b is adjuvant treatment).

[redacted]

Priming injections of EO2401 will be started as soon as possible after confirmation of disease progression; study baseline/screening MRI should be done no longer than 14 days before the start of treatment (MRI confirming progression of GB might be done earlier). The schedule of administration includes SC injections of EO2401 at 2-weekly intervals (4 times total) during the priming phase and then 4-weekly in the boosting phase starting from Week 10 (i.e., 4 weeks after the fourth administration of EO2401). The dose of EO2401 will depend on the outcome of Cohort 1, i.e., it can be either full dose [redacted], or half dose [redacted] Thus, Cohorts 2a and 2b should include full dose of EO2401 [redacted]

Nivolumab will be administered as an IV infusion at a dose of 3 mg/kg every 2 weeks in combination with EO2401 starting from the first priming injection. The nivolumab infusion is to start [redacted] after the EO2401 administration.

For all patients in Cohorts 2a and 2b, the general safety rules as described above applies, i.e., at related clinical Grade 4/5 AEs (the study treatment in the individual patient experiencing the event will be stopped for Grade 4 AEs) and 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.

At the global amendment 2 extended patient management measures are include for both Cohorts 2a and 2b (see **Section 6.3**).

After availability of safety and tolerability data for a 4-weeks period (2 administrations of EO2401 in combination with nivolumab, each followed by a 2-week observation period), in at least 3 patients of Cohorts 2 (the patients can be treated in either of the Cohorts 2a or 2b, respectively), the IDMC should convene and assess the totality of the safety data to recommend on the opening, or not, of Cohort 3.

Cohort 2c (see Figure 6B)

[redacted]

Cohort 2c is a cohort introduced by global amendment 2 (EOGBM1-18 version 3), to include 6 evaluable patients to assess safety and feasibility of a neoadjuvant/adjuvant treatment strategy including EO2401/nivolumab (both compounds started at the same time).

The treatment strategy includes the following important elements:

- The population includes patients assessed as candidates, and scheduled, for gross total resection of first recurrent GB, which can be safely postponed for 4-6 weeks according to local standards and treating physician's expertise.
- All screening measures per **Section 7.1** and **Section 7.2** should be followed; however, to shorten the time until planned surgery, the target for screening period #2 is 14 days (+/- 7 days). Note, due to patient specific circumstances the time window can be extended if deemed adequate by the treating physician after consultation with the Sponsor Medical Monitor.
- Neoadjuvant EO2401 (full dose SC, [redacted]) plus nivolumab (IV infusion at a dose of 3 mg/kg) administered twice with 2 weeks interval. The nivolumab infusion is to start [redacted] after the EO2401 administration.
    - All safety precautions and rules (e.g. treatment stopping rules) apply as for Cohorts 2a/2b.
    - **Table 1 Schedule of Assessments Cohort 2c** should be followed, and assessments and procedures as outlined in **Section 7.3** for V1 and V2 are also guiding.
    - [redacted]

- If there would be no possibility to perform surgery as planned after V2, the patient can still be evaluated for continued study treatment (from V3 and onwards) according to the same rules as for patients in Cohorts 2a/2b; e.g. if the MRI at V2 would show progression which does not allow surgery, but the patient does still not have significant symptoms (or symptoms can be controlled by symptom management, e.g. according to **Section 6.3**), study treatment may continue per the judgement of the treating physician until either confirmation of progression according to iRANO criteria, or establishment of PsPD. In the latter case, especially in situations of tumor shrinkage (no time limit), a delayed surgery can be performed, if in the best interest of the patient per the judgement of the treating physician and the patient concur (in such cases an individualized planning of study treatments should be done together with the Medical Monitor).
    - Note, patients who are not following the overall plan for neoadjuvant/adjuvant study treatment including the pre-planned surgery are not considered as part of the 6 evaluable patients, but the full available data from the patients will be analyzed.

- Planned surgery of recurrent disease:
    - Surgery, including associated treatments and measures, is a standard-of-care procedure outside of the clinical trial EOGBM1-18; it is indicated here to explain the treatment gap between the study procedures V2 and V3. Also note, surgery of recurrent glioblastoma is a highly individualized procedure and therefore exact timing cannot be stipulated; time window between V2 and V3 is assumed to be approximately 4-6 weeks.
    - The surgery as such including normal pre-, peri-, and post-operative care will be considered standard-of-care, and will only be documented in summary fashion in the CRF (e.g. date/time of surgery, type of procedure, outcome of procedure, length of primary hospitalization, reasons and lengths for possible further hospitalizations, and procedure related MRIs) and not considered AE/SAEs as long as the procedures are pre-planned or part of normal care/outcome of surgery for recurrent glioblastoma per judgement of the treating physician.
    - AEs, and when applicable per normal reporting rules SAEs, for events during the surgery period (time between V2 and V3, alternatively until the patient is declared to not be fit for further study therapy) will be reported for all events with a possible relationship to study drug treatment (irrespective of grade), for all surgery complications (irrespective of grade) which are not expected (per assessment by the treating physician), surgery complications ≥ Grade 3, and any events ≥ Grade 3.
    - In case a patient who has started study therapy cannot continue study therapy, e.g. study therapy in the adjuvant setting cannot be initiated after surgery, i.e. in case of study treatment discontinuation, a follow-up visit should be completed if at all possible (end-of-treatment visit including safety assessments per **Table 1**) approximately 30 days after completion of last dose of study treatment.
    - A key item is collection of tissue (for patients in Cohort 2c this is a mandatory consent item) from performed surgery for assessment of all applicable objectives and endpoints as outlined in **Section 2.1** and **Section 2.2**.

- Adjuvant EO2401 [redacted] plus nivolumab (IV infusion at a dose of 3 mg/kg) administered twice with 2 weeks interval, then switch to EO2401 on a 4-weekly schedule in the boosting phase, and continued nivolumab 2-weekly. The nivolumab infusion is to start [redacted] after the EO2401 administration.
    - All safety precautions and rules (e.g. treatment stopping rules) apply as for Cohorts 2a/2b.
    - **Table 1 Schedule of Assessments Cohort 2c** should be followed, and assessments and procedures as outlined in **Section 7.3** for V3 and V4 are also guiding, as are visits, assessments, and procedures for the treatment-boosting period outlined in **Section 7.4**.
- For all patients in Cohort 2c the general safety rules as described above applies, i.e., at related clinical Grade 4/5 AEs the study treatment in the individual patient experiencing the event will be stopped for Grade 4 AEs and 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.
- At the global amendment 2 extended patient management measures are included also for Cohort 2c (see **Section 6.3**).

Cohort 3 (see Figure 7)

[redacted]

[redacted]

Cohort 3 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 Cohorts 2a and 2b (see above). Cohort 3 will include administration of EO2401 in combination with nivolumab and bevacizumab from the start of treatment in the individual patients for assessment of safety, tolerability, immunogenicity, and preliminary efficacy of the combination. A total of 10 evaluable patients were planned for inclusion in Cohort 3 before global amendment 3 which increased the target recruitment to 26 patients.

Priming injections of EO2401 will be started as soon as possible after confirmation of disease progression; study baseline/screening MRI should be done no longer than 14 days before the start of treatment (MRI confirming progression of GB might be done earlier). The schedule of administration includes SC injections of EO2401 at 2-weekly intervals (4 times total) during the priming phase and then 4-weekly in the boosting phase starting from Week 10 (i.e., 4 weeks after the fourth administration of EO2401). The dose of EO2401 will depend on the outcome of Cohort 1, i.e., it can be either full dose [redacted] or half dose [redacted] Thus, Cohort 3 should include full dose of EO2401 [redacted]

Nivolumab will be administered as an IV infusion at a dose of 3 mg/kg every 2 weeks in combination with EO2401 starting from the first priming injection. The nivolumab infusion is to start [redacted] after the EO2401 administration.

Bevacizumab in Cohort 3 will regarding dose and schedule be used according to the US label for patients with recurrent GB.[69]

Bevacizumab will be administered as an IV infusion at a dose of 10 mg/kg every 2 weeks, in combination with EO2401/nivolumab. Bevacizumab will be administered [redacted] after the start of nivolumab administration per local practice at the study sites and in accordance with local labels for bevacizumab (e.g. regarding methods of administration, dilutions, etc.)[63, 69].

For all patients in Cohort 3, the general safety rules as described above applies, i.e., at related clinical Grade 4/5 AEs the study treatment in the individual patient experiencing the event will be stopped for Grade 4 AEs and 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.

At the global amendment 2 extended patient management measures are included generally for all Cohorts, i.e. also for Cohort 3, but the need for use of low-dose bevacizumab to treat tumor edema is assumed to be very limited (see **Section 6.3**).

3.2. Discussion of Study Design

This is a multicenter, Phase Ib/IIa, FIH study to assess the safety, tolerability, immunogenicity, and preliminary efficacy of EO2401 in patients with unequivocal evidence of progressive or first recurrent GB confirmed by MRI as defined by the RANO criteria (note: within the first 3 months after completion of radiotherapy and concomitant temozolomide, diagnosis of recurrence can be indistinguishable from PsPD on neuroimaging).[66]

The FIH protocol is an open-label multi-cohort study, intended to recruit a maximum of approximately 100 evaluable patients in total:

- Cohort 1 (as study safety lead-in, before global amendment 2) includes an evaluation by a 3-by-3 design of EO2401 monotherapy planned to be followed in the same individual patients by continued EO2401 in combination with nivolumab; 3 to 12 evaluable patients will be included depending on the safety profile of the administered treatments
    - In the safety lead-in part (3-by-3 design) of the trial, 3 evaluable patients started treatment in Cohort 1a, after which the safety lead-in was closed after a recommendation from the IDMC, and Cohorts 2a and 2b opened
    - In global amendment 2 (EOGBM1-18 version 3), plus via re-distribution of patients from Cohort 2b, 18 evaluable patients were added to Cohort 1a for further evaluation of the treatment schedule with a 4-weeks delayed start of nivolumab (safety precautions for the added patients will follow the same principles as for Cohorts 2a, 2b, 2c, and 3, since Cohort 1a is no longer a safety lead-in part of the trial) (recruitment finalized before global amendment 3)
- Cohorts 2a and 2b are an evaluation of EO2401 in combination with nivolumab planned to include approximately 48 evaluable patients in total
    - Before global amendment 2 (EOGBM1-18 version 3), 23 evaluable patients started treatment in Cohort 2a (after re-distribution of patients between Cohort 1 and Cohort 2a to keep a relevant safety database; see further **Section 1.2.3**)
    - In global amendment 2 (EOGBM1-18 version 3), plus via re-distribution of patients from Cohort 2b, 15 evaluable patients were added to Cohort 2a for further evaluation of the treatment schedule with simultaneous start of EO2401 and nivolumab, but with inclusion of extended patient management measures (recruitment finalized before global amendment 3)

  - Cohort 2b was planned to include 15 evaluable patients both before and after global amendment 2 (EOGBM1-18 version 3), but number of patients lowered to 6 by re-distribution of patients to Cohort 1a and 2a (recruitment finalized before global amendment 3)

- Cohort 2c is a cohort introduced by global amendment 2 (EOGBM1-18 version 3), to include 6 evaluable patients to assess safety and feasibility of a neoadjuvant/adjuvant treatment strategy including EO2401/nivolumab (both compounds started at the same time)
- Cohort 3 is an evaluation of EO2401 in combination with nivolumab and bevacizumab planned to include 10 evaluable patients before global amendment 3 which increased the target recruitment to 26 patients. [redacted]

The target population, patients with progressive or first recurrent GB or first recurrent disease, utilizing the selected inclusion/exclusion criteria, which are deemed appropriate by leaders in the field for an FIH trial of a novel immunotherapy approach, is limited; therefore, this study is planned to be conducted across approximately 10 sites.

There is clearly an unmet medical need for the selected patient population indicated by the very limited efficacy of current treatment approaches (see Section 1.3), and thus there is a great interest in continued research and to offer new treatment options to these patients.

In Cohort 1 (as study safety lead-in, before global amendment 2), an in-principle traditional 3-by-3 design is utilized for the initial safety and tolerability evaluation of the novel compound EO2401. However, since it has been clearly indicated for EO2401 in preclinical models that efficacy seems dependent on coadministration with CPIs, and similar findings can be found for other immunization approaches, it has been judged that an early combination of EO2401 with CPI is crucial for a relevant evaluation of the potential to induce adequate immunity in patients with established tumors (and also to give the patients some chance to notice any benefit from the treatment). Thus, after a short monotherapy period with EO2401, the individual patients, even in Cohort 1, are intended to continue treatment with the combination of EO2401 and CPI (i.e., nivolumab).

The more optimal treatment concept, i.e., as early as possible combination of EO2401 with CPI, is introduced from the start of Cohorts 2a and 2b where the start of the cohort is dependent on adequate safety in patients treated in Cohort 1. The difference between patients to be recruited to Cohorts 2a and 2b is governed by inclusion criterion #2 by which patients in Cohort 2a will have at least one measurable lesion and patients in Cohort 2b will not have any measurable enhancing disease. Practically, this means that patients to be recruited to Cohort 2b will have had successful secondary surgery for their post-primary treatment recurrent/progressive disease before inclusion into the trial. The reason to evaluate the trial treatment concept in both Cohorts 2a and 2b is that the outcome might be different in the two groups, and principles for how patients with first recurrent/progressed GB are treated are not globally uniform.

After safety has been assessed in the initial patients in Cohorts 2a and 2b, a further cohort, Cohort 3, is intended to open, including also the anti-angiogenic treatment bevacizumab, giving

the patients a further potential possibility for a prolonged treatment period with the EO2401 and CPI combination; it is expected that bevacizumab might prolong the time to progression and thereby allow an extended number of immunizations to be given. Of note is that the bevacizumab containing cohort is only going to include patients in the United States due to differences in the labeling of the drug in the United States and Europe.

Global amendment 2 of the protocol (leading to EOGBM1-18 version 3) includes also a Cohort 2c to assess safety and feasibility of a neoadjuvant/adjuvant treatment strategy which in other trials have shown interesting early signs of efficacy.[100, 101] Global amendment 2 also expanding the sample size of the trial and introducing extended patient management measures is described also in **Section 1.2.3**.

Thus, despite the limited efficacy of available treatments, some targeted therapies (e.g., CPI and angiogenesis inhibitors) which have shown some signs of efficacy in GB are utilized in the current trial to accomplish an early assessment of mainly safety and immunogenicity for EO2401 in different contexts. Considering the early nature of the trial, i.e., FIH, no control groups without administration of EO2401 will be included, but the information to be gained from the combinations might be crucial for the further planning of the development of EO2401 in GB.

Safety and tolerability are obviously the main assessment items in this FIH trial, and it is judged that the selected design has the possibility to both stringently evaluate early toxicity of EO2401 monotherapy (via the 3-by-3 design part in Cohort 1), but considering the current immunization approach also the long-term impact of induced immunity and the administered drugs (via the demand of thorough assessments of related Grade 3/4 AEs for all patients in all cohorts throughout all study treatments; general safety rules per Section 3.1).

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 trial conduct actions. The IDMC will be called on to assess related ≥ Grade 4 AEs in any patient at any time during the trial, or any medically important event as detected and proposed by the Investigators or the Sponsor. In addition, further monitoring by the IDMC is mandated in relation to the recruitment and treatment of patients in Cohort 1 (as study safety lead-in, before global amendment 2), and the IDMC will recommend on possible expansions in Cohort 1, and transition from Cohort 1 to 2a and 2b, and from Cohorts 2a and 2b to 3.

It is acknowledged that the trial is intensive regarding sampling, but considering that the main parameter for evaluation in the trial, beside safety, is to analyze EO2401-induced immunity and that the tests for such analyzes unfortunately demand relatively high volumes of material (e.g., PBMC), it is if the trial should be adequate and relevant to perform a necessity; a minimization strategy with regard to sampling has been applied, still without risking to get to a trial without an appropriate readout. The future development of the new compound EO2401 will be directed by the ability to analyze induced immunity adequately and thus this is a key aspect of the trial.

3.3. End of Study Definition

The end of the study is defined as the date of the last visit of the last patient in the study.

3.4. Medical Care of Patients After End of Study

The Investigator should ensure that the patients receive appropriate standard of care to treat the condition under the study.

3.5. Selection of Doses and Regimen in the Study

3.5.1. EO2401

In the landscape of the clinical studies conducted in peptide vaccination in the treatment of cancers, no standard vaccine administration scheme is established yet. Repeated administration of vaccine is described to augment the immune responses to the antigens[79] by expanding the pool T cells and allowing maintenance of the therapeutic response. In the cancer vaccine field, multiple injections have been shown to increase or at least maintain the number of specific CD8 T cells.[80,81]

To maintain an efficient immune response, EO2401 will be injected at 2-weekly intervals (4 times total) during the priming phase and then 4-weekly in the boosting phase starting from the final priming injection. Nonclinical immunogenicity experiments have demonstrated establishment of efficient immune response following bi-weekly injection (2 injections) in several HLA-A2 transgenic mice models.[62] Several ongoing clinical trials (NCT03047928,[82] NCT02455557,[83] NCT02737787,[84] NCT02826434,[85] NCT00003895,[86] and NCT01176461[87]) with peptide vaccine use a similar design based on a frequency of 2-weekly injections followed by boosting phase, supporting the choice of this regimen in the EOGBM1-18 trial.

The [redacted] to be injected to the patients was selected based on published clinical trial results (NCT01970358, A Phase I Study with Personalized NeoAntigen Cancer Vaccination in Melanoma[88]) and ongoing clinical trial (NCT02802943 iVAC-L-CLL01: Patient-individualized Peptide Vaccination in Combination With Lenalidomide After First Line Therapy of CLL; NCT01723813 Peptide Vaccinations Plus GM-CT-01 in Melanoma). Note that in the past, despite promising nonclinical results in animal models, the peptide-based cancer vaccination approach has not demonstrated unequivocal efficacy in patients. [redacted]

[redacted]

[redacted]These results support the use of UCP2 as helper peptide in the intended clinical trial with EO2401.

[redacted]

In addition, according to the recommendations of the Cancer Vaccine Clinical Trial Working Group,[89] no dose escalation was planned to be conducted in this trial.

All together these results support the design of the priming phase of the EO2401 Phase Ib/IIa clinical trial [redacted] the use of the helper peptide UCP2, and the regimen of 4 EO2401 administrations (300 μg of each peptide) during the 6 weeks in the priming phase.

3.5.2. Nivolumab

The recently completed Phase III CheckMate-143 study was the first randomized clinical trial in recurrent GB with the PD-1 CPI nivolumab as monotherapy administered at the dosage of 3 mg/kg.

Although it did not meet its primary endpoint of improved OS over bevacizumab monotherapy, nivolumab may still have a place in the successful treatment of this disease, potentially as combination therapy, as a small subset (8%) of patients in the CheckMate-143 study did

respond to nivolumab, and with a much longer DoR (11 months) than seen with bevacizumab therapy (5.3 months).[58, 98]

In the initial development, the dosage for the treatment of the other cancers for which nivolumab has obtained the marketed authorization was usually 3 mg/kg.

In this Phase Ib/IIa study, nivolumab is to be administered at the dosage of 3 mg/kg as IV infusion every 2 weeks.

3.5.3. Bevacizumab for continuous combination treatment in Cohort 3 – in US Only

Bevacizumab will regarding dose and schedule be used according to the US label for patients with a recurrent GB.[69]

The recommended dosage in the setting is 10 mg/kg every 2 weeks, in Cohort 3 for continuous treatment in this Phase Ib/IIa study, bevacizumab is administered at this frequency and at this dosage as an IV infusion.

Note, global amendment 2 (leading to EOGBM1-18 version 3) includes allowance for extended patient management measures of tumor site edema for all cohorts, and one component of these measures is low-dose bevacizumab; i.e. bevacizumab might also be used as symptomatic therapy in Cohorts 1a, 2a, 2b, and 2c, according to the rules outlined in **Section 6.3**.

4. SELECTION OF STUDY POPULATION

Only patients who meet all of the inclusion criteria and none of the exclusion criteria will be eligible to be recruited for this study. Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

4.1. Inclusion Criteria

To be eligible to participate in the study, patients must meet the following criteria:

1. Patients with unequivocal documented (including histological confirmation of GB at the primary diagnosis) first progression/recurrence of GB on MRI, as defined by RANO criteria
2. Patients with:
   - for Cohorts 1, 2a, and 3: at least 1 measurable lesion
   - for Cohort 2b: no measurable enhancing disease (defined as less than 1x1 cm in maximum bi-perpendicular plane)
   - for Cohort 2c: documented recurrence of GB deemed to be candidate for surgery as standard-of-care at the local institution, and for which the resection can safely be postponed for 4-6 weeks per local institutional guidance and treating physician judgement). In addition, for inclusion in Cohort 2c the patient must consent to mandatory collection of tissue samples from the time of diagnosis (if logistically available), and the planned surgery after neoadjuvant study therapy (see also **Section 7.1.1**).
     - [redacted]
3. Patients with an age ≥ 18 years old
4. Patients who are HLA-A2 positive
5. Patients with an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 or Karnofsky performance status ≥ 70 (see Appendix 3)
6. Patients should have received standard primary therapy including surgery (biopsy, incomplete or complete resection), radiation, temozolomide, if applicable
   a. Radiation therapy must have been finished at least 28 days before first study treatment administration
   b. Patients who received temozolomide as adjuvant therapy must have stopped the treatment and have a wash-out period of at least 28 days before first study treatment administration (6 weeks for nitrosoureas and at least 4 weeks, or 5 half-lives if longer, for experimental therapies, if this type of therapies have been included as components of adjuvant therapy)
   c. Patients with unmethylated methylguanine-DNA-methyltransferase (MGMT) promoter can be included even if they have not received temozolomide prior to the inclusion in this clinical study. [redacted]

[redacted]

7. Female patients of childbearing potential must have a negative serum pregnancy test within 72 hours prior to dosing
8. Considering the embryofetal toxicity of the nivolumab shown on 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 included:
        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
        iv. Intrauterine hormone-releasing system
        v. Bilateral tubal occlusion
        vi. ectomized partner
        vii. Sexual abstinence.

    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 8 months after the last treatment dose administered. Males must ensure that their partners of childbearing potential use highly effective contraception also
9. Patients having received the information sheets and who have provided written informed consent prior to any study-related procedures
10. Patients willing and able to comply with the scheduled visits, treatment plan, laboratory tests, and other study procedures indicated in the protocol.

4.2. 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 the first EO2401 administration, unless required to treat an AE

   [redacted]

2. Patients treated with radiotherapy, and cytoreductive therapy within 28 days (6 weeks for nitrosoureas) before the first EO2401 administration. In addition, patients should not have received any prior treatment with compounds targeting PD-1, PD-L1, CTLA-4, or similar compounds where general resistance against therapeutic vaccination approaches might have developed; patients should not have received systemic anti-tumor treatment or radiotherapy for their progressive or first recurrent GB
3. Patients with tumors primarily located in the infra-tentorial segment
4. Patients with known radiological evidence of extracranial metastases
5. Patients with presence of new intracranial hemorrhage (excluding, stable Grade 1) or uncontrolled seizure
6. Patients with significant leptomeningeal disease
7. Patients with abnormal (≥ Grade 2 NCI-CTCAE v5.0) laboratory values for hematology, liver, and renal function (serum creatinine). In detail, the following values apply as exclusion criteria:

   [redacted] laboratory values judged by the treating physician as clinically non-relevant

8. For patients who are planned to receive bevacizumab:
   a. Patients with nephrotic syndrome

b. Patients with proteinuria ≥ 2g/24 hours
c. Patients with history or active gastrointestinal perforation and fistula
d. Significant surgical procedure in the 4 weeks preceding the start of treatment or planned surgery
e. Unhealed wound
f. Patient with recent (4 weeks) history of hemoptysis of ½ teaspoon or more of red blood
g. Thrombotic episode within 6 months
h. Uncontrolled diabetes mellitus or hypertension
i. Posterior Reversible Encephalopathy Syndrome

9. Patients with persistent Grade 3 or 4 toxicities (according to NCI-CTCAE v5.0). [redacted]
10. Patients with contraindication to contrast-enhanced MRI
11. Other malignancy or prior malignancy with a disease-free interval of less than 3 years except those treated with surgical intervention and an expected low likelihood of recurrence such as basal cell or squamous cell skin cancer, or carcinoma in situ. Patients with adequately treated basal cell or squamous cell skin cancer, or carcinoma in situ are eligible
12. Patients with clinically significant active infection, cardiac disease, significant medical or psychiatric disease/condition that, in the opinion of the Investigator, would interfere with the evaluation of EO2401 or interpretation of patient safety or study results or that would prohibit the understanding or rendering of informed consent (i.e. only consent able patients can be enrolled in the study) and compliance with the requirements of the protocol – including (but not limited to):
    a. Bacterial sepsis or other similarly severe infections
    b. New York Heart Association > Grade 2 congestive heart failure within 6 months prior to study entry (see Appendix 4)
    c. Uncontrolled or significant cardiovascular disease, including:
        i. Myocardial infarction within 6 months prior to obtaining informed consent
        ii. Uncontrolled/unstable angina within 6 months prior to obtaining informed consent
        iii. Diagnosed or suspected congenital long QT syndrome
        iv. Any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or Torsades de pointes).
    d. Stroke within 6 months prior to obtaining informed consent
    e. Concurrent neurodegenerative disease
    f. Dementia or significantly altered mental status.

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

[redacted]

14. Patients with a history of solid organ transplantation or hematopoietic stem cell transplantation
15. Patients with a history or known presence of tuberculosis
16. Pregnant and breastfeeding patients
17. Patients with a history or presence of human immunodeficiency virus (HIV) and/or active hepatitis B virus (HBV)/hepatitis C virus (HCV)
18. Patients who have received live or attenuated vaccine therapy used for prevention of infectious diseases including seasonal (influenza) vaccinations within 4 weeks of the first dose of study drug
19. Patients with a history of hypersensitivity to any excipient present in the pharmaceutical form of investigational medicinal product (IMP)
20. Patients under treatment with immunostimulatory or immunosuppressive medications, including herbal remedies, or herbal remedies known to potentially interfere with major organ function
21. Patients with known drug and alcohol abuse
22. Patients with known or underlying medical or psychiatric condition that, in the Investigator's opinion, would make the administration of study drug hazardous to the patient or obscure the interpretation of toxicity determination or AEs
23. Patients who have received treatment with any other investigational agent, or participation in another clinical trial (clinical trial including active interventions are prohibited; participation in clinical trials for data collection purposes only are permitted) within 28 days prior to first study treatment administration and during the treatment period. Note, for investigational agents there should be a wash-out period of at least 28 days, or 5 half-lives if longer, before first study treatment administration
24. Patients deprived of their liberty or under protective custody or guardianship. [redacted]

4.3. Discontinuation Criteria

4.3.1. Screen Failures

The trial will include a 2-stage consent and screening procedure (see Sections 7.1 and 7.2). The first minimized consent, and screening period #1, is related to the procedure of HLA-testing, and before testing also establishing based on available information that the patient has eligible glioblastoma and an age ≥ 18 years. The second stage of the consent procedure, and screening period #2, will be related to all other procedures needed before being included into the trial. 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.

Screen failures are defined as patients who consent to participate in the clinical study but are not subsequently included in the analysis population due to failure of the eligibility criteria. A minimal set of Screen failure information is required to ensure transparent reporting of Screen failure patients to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, Screen failure details, eligibility criteria, and any SAEs.

Patients who are HLA-A2 negative will have a "Screen failure" status. HLA-A2 testing will be performed locally.

Patients who have laboratory abnormalities may be rescreened at the discretion of the Investigator.

4.3.2. Discontinuation of Study Treatment and Patient Discontinuation

The Investigator has the right to discontinue a patient from study treatment or withdraw a patient from the study at any time after discussing it with the Sponsor. In addition, patients have the right to voluntarily discontinue study treatment or withdraw from the study at any time at his/her request for any reason. In instances where consent is withdrawn, the Investigator must clarify whether the patient is willing to continue to be followed (i.e., for survival).

Patients who discontinue study treatment prematurely will be asked to return to the clinical for follow-up assessments (see Section 7.4.5). The primary reason for premature study drug treatment discontinuation should be documented on the appropriate electronic Case Report Form (eCRF).

Reasons for discontinuation of study treatment may include, but are not limited to:

- Confirmed tumor/disease progression
- Any medical condition that the Investigator or Sponsor determines may jeopardize the patient's safety if he/she continues trial treatment
- AEs requiring discontinuation
- Pregnancy
- Major protocol deviation (i.e., affecting the patient's safety)
- Investigator or Sponsor determines it is in the best interest of the patient
- Patient noncompliance to study scheduled visits and assessments according to the Investigator
- Patient withdrawal of consent to receive further study treatment.

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

- Patient withdrawal of consent to be followed up
- Patient lost to follow-up
- Study termination by the Sponsor or regulatory authorities.

4.3.3. Discontinuation of Study Treatment

Patients who discontinue study treatment prematurely will be asked to return to the clinic for follow-up assessments (see Section 7.4.5). The primary reason for premature study drug treatment discontinuation should be documented on the appropriate eCRF.

A patient must be withdrawn from the study for the following reasons:

- Withdrawal of informed consent
- If IDMC request study discontinuation due to an SAE/AE
- For childbearing females, confirmation of pregnancy
- Confirmed tumor/disease progression.

A patient may be withdrawn for the following reasons:

- Protocol violations that may impact the outcome of the study
- Investigator's discretion
- Sponsor or regulatory authorities' decision.

4.3.4. 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 methods). These contact attempts should be documented in the patient's medical record and in the eCRF.

4.3.5. Follow-up of Patients Prematurely Discontinued from the Study Treatment Regimen or Withdrawn from Study

Every effort should be made to obtain information on patients who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. Patients will not be followed for any reason after informed consent has been withdrawn, although date of death should be recorded, where applicable and possible to retrieve.

Patients who discontinue from the study treatment regimen will be asked to complete the final study procedures (see Section 7.4.5), or if the patient is unable to attend a study visit, then a follow-up telephone call will take place. All AEs/SAEs that are ongoing at the time of discontinuation, or that develop prior to the final scheduled follow-up telephone call, will be followed for 30 days, or until resolution or stabilization.

4.4. 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. Study sites will be closed upon study completion. 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.

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

- AEs unknown to date (i.e., not previously reported in any similar investigational study drug trial with respect to their nature, severity, and/or duration)
- Increased frequency and/or severity and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined as baseline signs and symptoms)
- Medical or ethical reasons affecting the continued performance of the study
- Difficulties in the recruitment of patients
- Cancellation of drug development.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for the early closure of a study site by the Sponsor or Investigator 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
- Inadequate recruitment of participants by the Investigator.

5. STUDY TREATMENTS

5.1. Treatments Administered

[redacted]

Cohort 1

In the priming phase, patients included in sub-cohorts 1a and 1b will receive an SC injection of [redacted] EO2401 [redacted] every 2 weeks for a total of 4 injections. This will be followed by a boost phase with 1 administration every month starting 4 weeks after the final priming injection and continued until confirmed tumor progression, intolerable toxicity, death, Investigator or patient decision, or early termination of the study at the request from the Sponsor, where study treatment will be stopped and appropriate standard of care will be initiated by the Investigator.

In sub-cohorts 1a and 1b, the full dose of EO2401 [redacted] will be administered and in sub-cohorts 1c and 1d it is a half dose that will be administered [redacted]

- In a rotating way by injection (so an injection site will be used every fourth time), with the injection sites being right and left upper extremity and right and left lower extremity/inguinal area, with the more specific locations being:
  - For upper extremity = SC injection in the area of armpit, or deltoid muscle of arm, depending on the constitution of the patient
  - For lower extremity = SC injection in the area of groin, or anterolateral thigh muscle, depending on the constitution of the patient.

The CPI nivolumab will start at the third priming injection (i.e., at Week 4) as an IV infusion at a dose of 3 mg/kg every 2 weeks in combination with EO2401. Implantation of a port-a-cath can be considered by the site to administer nivolumab.

For further information on administration of nivolumab, refer to the European SmPC or the US PI for nivolumab.[59,60]

EO2401 will be injected [redacted] from the start of the reconstitution.

EO2401 will be administered first followed by nivolumab, [redacted]

Cohorts 2a and 2b

The dose of EO2401 will be determined in Cohort 1 (as study safety lead-in, before global amendment 2) and [redacted] given as an SC injection at 2-weekly intervals (4 times total) during the priming phase and then 4-weekly starting from the final priming injection in

the boosting phase until progression, death, Investigator or patient decision, or early termination of the study, where study treatment will be stopped and appropriate standard of care will be initiated by the Investigator.

[redacted]

Nivolumab will be administered as an IV infusion at a dose of 3 mg/kg every 2 weeks in combination with EO2401 starting from the first priming injection. Implantation of a port-a-cath can be considered by the site to administer nivolumab.

For further information on administration of nivolumab, refer to the European SmPC or the US PI for nivolumab.[59,60] EO2401 will be administered first followed by nivolumab, [redacted]

Cohort 2c

Neoadjuvant EO2401 [redacted] plus nivolumab (IV infusion at a dose of 3 mg/kg) will be administered twice with 2 weeks interval.

There will be a study-specific MRI at the day of the second dose of EO2401/nivolumab (V2) to ensure that the tumor is still suited for the intended surgery.

After planned surgery of recurrent disease and adequate recovery, adjuvant EO2401 [redacted] plus nivolumab (IV infusion at a dose of 3 mg/kg) will be administered twice with 2 weeks interval, then switch to EO2401 on a 4-weekly schedule in the boosting phase and continued nivolumab 2-weekly.

The nivolumab infusions are to start [redacted] after the EO2401 administration.

The specifics of Cohort 2c are outlined in Section 3.1, and for further information on administration of nivolumab, refer to the European SmPC or the US PI for nivolumab.[59,60]

Cohort 3

[redacted]

Cohort 3 is planned to be initiated after a review of the safety and tolerability data from Cohorts 2a and 2b (see Section 3.1), and will include administration of EO2401 in combination with nivolumab and bevacizumab from the start of treatment in the individual patients.

EO2401 and nivolumab administration schedules and doses are the same as in Cohorts 2a and 2b.

Bevacizumab will be administered as an IV infusion at a dose of 10 mg/kg every 2 weeks in combination with EO2401 and nivolumab.

The sequence of administration will be starting with EO2401, followed [redacted] later the infusion of nivolumab, which will be followed [redacted] by the bevacizumab administration.

For further information on administration of nivolumab and bevacizumab, refer to the US PIs and EU SmPCs for nivolumab and bevacizumab, respectively.[59,60,63,69]

5.2. Investigational Medicinal Product (EO2401)

5.2.1. Pharmaceutical Form

The IMP of the study, EO2401, [redacted] containing 4 peptide drug substances [redacted]
The final concentration of each peptide is 300 µg/mL.

Further details are provided in the EO2401 Investigator Brochure.[62]

5.2.2. Preparation of the Formulation

The IMP is provided to the clinical sites [redacted]

[redacted]

[redacted]

[redacted]

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

[redacted]

[redacted]

5.2.3. Administration

EO2401 will be injected [redacted] from the start of the reconstitution. Each SC injection must be administered at different sites as described in Section 5.1.

5.2.4. Packaging and Labeling

The final emulsified EO2401 DP in adjuvant will be reconstituted by the investigational site pharmacy, following instructions described in the Pharmacy Manual.

Adequate supplies of the study will be provided to each investigational site for reconstitution.

EO2401 will be manufactured as per Good Manufacturing Practice (GMP) and supplied by the Sponsor as a Mixing Kit consisting of 2 component glass vials plus additional material.

[redacted]

For all vials, a system in accordance with GMP will be used; ensuring each dose of EO2401 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.

5.2.5. 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 a Mixing Kit, in 2 boxes:

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

5.4. Nivolumab

5.4.1. Pharmaceutical Form

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

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

Refer to the SmPC and/or US PI for nivolumab. [59,60]

5.4.2. Administration of Infusion

EO2401 will be administered first followed by nivolumab, [redacted] The prescribed dose for this study is 3 mg/kg every 2 weeks in combination with EO2401.

Nivolumab infusion must not be administered as an IV push or bolus injection. Administer the nivolumab infusion IV over a period of 30 or 60 minutes depending on the dose, or according to local standard practice. The infusion should not be infused at the same time in the same IV line with other agents. A separate infusion line for the infusion should be used.

An infusion set and an in-line, sterile, non-pyrogenic, low protein binding filter (pore size of 0.2 to 1.2 μm) should be used. Nivolumab infusion is compatible with PVC and polyolefin containers, glass bottles, PVC infusion sets, and in-line filters with polyethersulfone membranes with pore sizes of 0.2 to 1.2 μm.

Nivolumab will be co-administered starting with the third priming injection in Cohort 1 and from the beginning of the priming phase in Cohorts 2 and 3.

After administration of the nivolumab dose, flush the line with sodium chloride 9 mg/mL (0.9%) solution for injection, or 50 mg/mL (5%) glucose solution for injection, or according to local standard practice. Refer to the SmPC and PI for nivolumab. [59 ,60]

Implantation of a port-a-cath can be considered by the site to administer nivolumab.

Each mL of this medicinal product contains 0.1 mmol (or 2.5 mg) sodium, this should be taken into consideration when treating patients on a controlled sodium diet.

5.4.3. Package and Labeling

Nivolumab (ready-to-use) will be obtained by commercial sources and clearly labeled for use.

Adequate supplies of nivolumab in 4 and 10 mL glass vials will be provided to each investigational site for preparation and infusion. The vial stopper will be a coated butyl rubber with a grey aluminum flip-off seal.

A detailed process for handling and administration of nivolumab will be specified in the SmPC.[59]

5.4.4. Storage and Stability

Nivolumab should be stored in the refrigerator at 2°C to 8°C in its original package in order to protect from light. Once opened, the medicinal product should be infused or diluted, and infused immediately.[59,60]

If not used immediately, chemical and physical in-use stability of nivolumab has been demonstrated for 24 hours at 2°C to 8°C protected from light and a maximum of 8 hours at 20°C to 25°C with room light (this 8-hour period of the total 24 hours should be inclusive of the product administration period).

5.5. Bevacizumab

Bevacizumab at the full labelled dose in the US (US PI[69]) will be administered to patients recruited to Cohort 3 at sites in the United States. The compound will be prescribed by the responsible physician at the site in the United States. All procedures for Cohort 3 regarding bevacizumab administration at sites in the USA will be according to local site accepted documented standards or the US PI.[69]

After implementation of global amendment 3 (to protocol version 4) sites in Europe will also be allowed (provided all applicable authority approvals are received) to recruit patients to Cohort 3.

Bevacizumab for use at European sites will be considered as an IMP and will consequently be obtained by the Sponsor via commercial sources and clearly labeled for use. Adequate supplies of bevacizumab will be provided to each European investigational site for preparation and infusion. A detailed process for handling and administration of bevacizumab is specified in the European SmPC.[63] All procedures for Cohort 3 regarding bevacizumab administration at sites in Europe will be according to local site accepted documented standards or the European SmPC.[63]

5.5.1. Pharmaceutical form

Bevacizumab is a concentrate for solution for infusion containing 25 mg/ml of bevacizumab.

Generally, bevacizumab must not be administered or mixed with dextrose solution. The necessary amount of bevacizumab should be withdrawn and diluted to the required administration volume with sodium chloride 9 mg/ml (0.9%) solution for injection. The concentration of the final bevacizumab solution should be kept within the range of 1.4 mg/ml to 16.5 mg/ml. In the majority of the occasions the necessary amount of bevacizumab can be diluted with 0.9 % sodium chloride solution for injection to a total volume of 100 mL.

Bevacizumab first infusion IV will be administered according to local standard practice.

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

Bevacizumab will be administered as an IV infusion at a dose of 10 mg/kg every 2 weeks in combination with EO2401 and nivolumab.

Bevacizumab will be administered [redacted] after the start of nivolumab administration per local practice.

Low-dose bevacizumab, implemented by the global amendment 2 (leading to EOGBM1-18 version 3), may be used for management of tumor edema in all cohorts *per* the specific initiation, dose, time, and duration description in **Section 6.3**. All procedures for administration of bevacizumab also at a low-dose will be according to local site accepted documented standards or the SmPC or US PI. [63, 69]

5.5.2. Storage and stability

Bevacizumab must be stored in refrigerator (+2°C to +8°C) in the outer carton in order to protect from light.

Once diluted, the chemical and physical in-use stability has been demonstrated for 30 days at +2°C to +8°C plus an additional 48 hours at +2°C to +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.

5.6. Study Treatment Accountability, Reconciliation, and Return

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 not authorized by the Sponsor to be destroyed at the site.

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.

5.7. Study Treatment Handling and Disposal

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 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.

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.

Further guidance and information for the final disposition of unused study treatment are provided in the pharmacy binder.

5.8. Method of Treatment Assignment

This is an open-label, nonrandomized trial and each newly enrolled patient will be assigned a unique patient identification number and assigned to the treatment after approval of eligibility, where applicable, on a first-come first-served basis. Slot assignments will be managed via a cohort management plan.

[redacted]

Study treatment will be dispensed at the study visits summarized in the Schedule of Assessments (Table 1A and Table 1B).

Patients evaluable for safety and AEs will include all patients who have received at least 1 dose of EO2401. Patients who are not considered evaluable for safety will be replaced. Patients evaluable for efficacy based on the PP population will consist of any patients who received at least [redacted] (EO2401 alone or EO2401 in combination with CPI nivolumab or nivolumab/bevacizumab) for whom no important protocol deviations occurred and have at least 1 evaluable post-Screening tumor assessment. Patients who are not considered evaluable for the PP population will not be replaced and be included in the FAS.

Patients who are not considered evaluable for safety will be replaced and given a new identification number. Patients will be replaced until the desired number of evaluable patients is achieved in this study. The patient's inclusion log will be filled in with patients that are included in the study but not considered as evaluable.

5.8.1. Dose Modifications, Permanent Discontinuation of Treatment, or Treatment Delay

5.8.1.1. Dose Reductions

The only adjustments regarding dose for the EO2401 combination partners nivolumab and bevacizumab are withholding of 1 or several doses, or discontinuation of treatment; such measures should be taken in accordance with the labeled recommendations in the European SmPC [59,63] or the US PI[60,69], which are available for both compounds. Dose adjustments for EO2401 are also including the potential of withholding one or several doses (see **Section 7.6.1.1**), or discontinuation of treatment. In addition, a lowered dose might be applied in the potential transition from sub-cohort 1b to sub-cohort 1c in relation to safety events (the dose of EO2401 will then be halved).

5.8.1.2. Treatment Delay

Dosing delay or discontinuation for nivolumab and/or bevacizumab may be required based on individual safety and tolerability. Guidelines for permanent discontinuation or withholding of doses are described in Table 6 in Section 7.6.1.1.

5.8.1.3. Treatment Discontinuation

For patients receiving a combination of EO2401 + nivolumab or EO2401 + nivolumab + bevacizumab, the treatment will continue according to schedule with EO2401 (and bevacizumab when applicable) even if nivolumab is temporarily withheld or discontinued, and likewise the treatment will continue according to schedule with EO2401 (and nivolumab when applicable) even if bevacizumab is temporarily withheld or discontinued. If EO2401 would be stopped, the other drugs in the combination will also be stopped in the context of being trial treatment. In such cases, the Investigator responsible for the patient in question will advise on adequate further treatment options.

5.9. Blinding

This is an open-label study; blinding procedures are not applicable.

5.10. Treatment Compliance

Since all treatments are administered at the clinical site by site personnel, the Investigator or designee must maintain accurate records of all study treatments, including dates of study drug receipt, quantities received and dispensed, and batch/lot numbers. In addition, the study treatment must be noted in the patient's medical records and eCRF, with the date and time of administration and dose of each study treatment.

6. PRIOR AND CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1. Prior and Concomitant Therapy

The Investigator must record the use of all prior and concomitant medications or vaccines taken during 30 days prior to signing informed consent and for the duration of the study both prescribed and over-the-counter, including herbal remedies in the source documents and eCRF along with:

- Reasons for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency.

In case of medical need for a new surgical resection, all details must be collected in the eCRF.

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.

See **Section 3.1** regarding additional documentation requirements for concomitant therapies in Cohort 2c in relation to planned surgery.

6.2. Prior and Concurrent Illness

The Investigator should document all prior significant illnesses that are relevant to patient safety or that the patient has experienced prior to Screening.

Additional 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.

6.3. Permitted Concomitant Medications

All noncancer therapies that the responsible physician feels are appropriate are allowed in this study, except for the medications listed below in Section 6.5.

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

In case of infusion-related reactions after the first patient is enrolled, administration of paracetamol and/or antihistaminic drugs is acceptable. Please refer to Appendix 7.

Patients should receive full supportive care during and after the administration of EO2401 injection and nivolumab or nivolumab/bevacizumab or standard of care treatment per local practice and according to the judgment of the Investigator or treating physician.

The use of systemic corticosteroids and other immunosuppressants at baseline, before starting nivolumab, should be avoided because of their potential interference with the pharmacodynamics activity. However, systemic corticosteroids and other immunosuppressants can be used after starting nivolumab to treat immune-related adverse reactions (see Section 7.6.1.1). Dosage, frequency, dates of administration, and route of administration must be recorded in the dedicated eCRF pages. Preliminary results show that systemic immunosuppression after starting nivolumab treatment does not appear to preclude response on nivolumab.

Nivolumab should not be resumed while the patient is receiving immunosuppressive doses of corticosteroids or other immunosuppressive therapy. Prophylactic antibiotics should be used to prevent opportunistic infections in patients receiving immunosuppressive therapy.[60]

[redacted]

[redacted]

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 Grade 3 or 4 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 (EO2401 administered every 4 weeks), 2 weeks after the latest EO2401 administration (and

thereby 2 weeks before the next EO2401 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 time interval between administrations of a COVID-19 vaccine and nivolumab is recommended to be at least 2 days before the nivolumab administration during the boosting phase (EO2401 administered every 4 weeks) and in a different location. An alternative option, for patients in EOGBM1-18 and being in the boosting period could also be to not administer one dose of nivolumab (in this case the visit has to be run as planned to obtain the safety laboratory data sampling documented and the lack of nivolumab administration has to be related to the COVID-19 vaccination in the eCRF).

- The currently (2021-03-24) by US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved COVID-19 vaccines are acceptable for use in the trial (local restrictions per country specific authority of course takes precedence):

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

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

6.4. Contraception

Where sexually active, female patients of childbearing potential, or male patients with reproductive capacity and partners of childbearing potential, must be willing to use an acceptable method of contraception (see inclusion criteria) from the signing of the ICF through 6 months (female)/8 months (male) after receiving the last dose of study drug. Acceptable contraception methods include a sterile sexual partner, hormonal contraceptives (oral, injection, transdermal patch, or implant), or intrauterine device. Refer to the inclusion criterion in Section 4.1 for a list of highly effective contraception.

Postmenopausal status will be confirmed with a Screening serum follicle-stimulating hormone (FSH) level > 40 mIU/mL.

6.5. Prohibited Concomitant Medications

Patients are not permitted to take those medications specified in the exclusion criteria (Section 4.2), and the following below:

- Other investigational agents or participate in a device study within 28 days prior to enrollment or receive systemic anticancer therapy within 28 days and during the study, and will make best efforts not to start any other investigational product or device study within 30 days after last drug administration.
    - Note, for investigational agents there should be a wash-out period of at least 28 days, or 5 half-lives if longer, before first study treatment administration.
- Dexamethasone > 2 mg/day or equivalent (i.e., 13 mg/day of prednisone) within 14 days before the first EO2401 administration, unless required to treat an AE

The following are not permitted from 28 days prior to and during study drug administration:

- Treatment with PD(L)-1, immunotherapy, radiotherapy, and/or any other investigational agents (does not apply to the defined study medications nivolumab and bevacizumab)
    - Note, patients should not have received any prior treatment with compounds targeting PD-1, PD-L1, CTLA-4, or similar compounds where general resistance against therapeutic vaccination approaches might have developed.
- Other systemic anticancer therapy within 28 days or 5 half-lives if longer, prior to study entry, whichever is shorter
- Live or attenuated vaccines
- Immunostimulatory or immunosuppressive medications, including herbal remedies, or herbal remedies known to potentially interfere with major organ function

Note, the use of high dose dexamethasone has been incorporated as part of measures to treat significant neurological symptoms, see **Section 6.3**, and such treatment is thereby not prohibited.

7. STUDY ASSESSMENTS AND PROCEDURES

Study procedure and their timings are summarized in the Schedule of Assessments for Cohorts 1, 2 and 3 (Table 1). A visit window of ± 2 days, if nothing else stated in relation to Table 1, has been included in this study. As protocol waivers or exemptions are not allowed, with the exception of immediate safety concerns, these should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the patient should continue or discontinue the study drug. Adherence to the study design requirements, including those specified in the Schedule of Assessments, is essential and required for study conduct.

[redacted]

7.1. Screening Period #1

The trial will include a 2-stage consent and screening procedure.

The first minimized consent, and screening period #1, is related to the procedure of HLA-testing, and before testing also establishing based on available information that the patient has eligible glioblastoma and an age ≥ 18 years.

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 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. genotyping of the HLA-A loci 2 to 2 digits) is considered satisfactory for trial enrolment. However, 4-digit resolution typing is preferred; also, a more complete HLA-typing is preferred.

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.

7.2. Screening period #2

The second stage of the consent procedure, and screening period #2, will be related to all other procedures, beside those described in Section 7.1, needed before being included into the trial. 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 Screening evaluations must be completed and reviewed within the timeframe defined in the Schedule of Assessments (Table 1) to confirm that potential patients meet all eligibility

criteria. The Investigator will maintain a Screening log to record details of all patients screened and to confirm eligibility or record reasons for Screen failure (Section 4.3.1), as applicable.

Procedures conducted as part of the patient's routine clinical management (e.g., blood count) and obtained before signing the consent form may be utilized for Screening purposes, provided the procedure met the protocol-specified criteria and was performed within the timeframe defined in the Schedule of Assessments (Table 1).

All Screening laboratory tests will be performed locally. The brain MRI will be performed and read at the local site. The maximum amount of blood from each patient 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 that fail Screening will be replaced.

The following will be conducted/collected and all results will be documented during Screening:

- Informed consent
- Eligibility criteria assessment according to inclusion/exclusion criteria
- Demographics (including weight and height) and medical history to include oncologic history, demographics, history of other disease processes (active or resolved), and concomitant illness
- IDH and MGMT status will be collected
- HLA-A2 positivity confirmation (patients who are HLA-A2 negative will fail Screening). HLA-A2 testing will be performed locally
- Collection of all previous chemotherapy and radiotherapy treatments
- ECOG or Karnofsky performance status
- Complete physical examination of major body systems
- Vital signs (including heart rate [HR], blood pressure [BP], temperature, and respiratory rate)
- 12-lead electrocardiogram (ECG; performed as a triplicate trace within 2 months before Screening)
- [redacted]
  - [redacted]
- Brain MRI (MRI should be done within 14 days before the start of treatment)
- NANO scale/questionnaire completion to assess neurological function
- Assessment of AEs/toxicity
- Assessment of prior and concomitant medications

- Clinical laboratory assessments (hematology, biochemistry, electrolytes, coagulation, hepatic, and renal function; see Section 7.7). All laboratory tests will be performed locally. HIV, HBV, HCV, s-cortisol (and ACTH when applicable; if the level of serum-cortisol is below the lower local normal limit, an ACTH stimulation test should be performed), TSH and free T4, will be assessed within 28 days prior first intake of study medication. A polymerase chain reaction viral detection test will also be used to confirm HCV status when a person has immunodeficiency as this condition can be associated with a false negative HCV antibody test result.

  [redacted]

- Complete urinalysis
- Pregnancy test will be performed for all female patients of childbearing potential. A serum pregnancy test will be performed 72 hours prior to start of treatment, if applicable. Postmenopausal status for postmenopausal females will be confirmed with a Screening serum FSH level > 40 mIU/mL
- Blood collection [redacted]
- [redacted]

7.3. Treatment Priming Phase

7.3.1. Week 0 (Visit 1)

Visit 1 must be performed within 14 calendar days from baseline MRI investigation ± 2 days.

Before the first administration of the treatment, the inclusion and exclusion criteria must be checked by the Investigator.

The following assessments will be conducted:

- Clinical laboratory assessments (hematology, biochemistry, coagulation, hepatic, and renal function, electrolytes). All laboratory tests will be performed locally.
- Complete urinalysis
- Administration of EO2401. [redacted]

  [redacted]

- Infusion of nivolumab for Cohorts 2a and 2b and Cohort 3

- Infusion of bevacizumab only for Cohort 3
- Demographics, except height
- ECOG or Karnofsky performance status
- Physical examination
- Vital signs
- Assessment of AEs/toxicity
- Assessment of prior and concomitant medications
- Blood collection [redacted]
- [redacted]

7.3.2. [redacted]

The following assessments will be conducted:

- Clinical laboratory assessments [redacted]
- Complete urinalysis
- Administration of EO2401
- Infusion of nivolumab for Cohort 1 (for Visit 3 only), Cohorts 2a and 2b, and Cohort 3
- Infusion of bevacizumab only for Cohort 3
- Demographics, except height
- ECOG or Karnofsky performance status
- Physical examination
- Vital signs
- Assessment of AEs/toxicity
- Assessment of prior and concomitant medications
- Pregnancy test (for Visit 2, if applicable)
- Blood collection [redacted]

7.3.3. [redacted]

The following assessments will be conducted:

- Clinical laboratory assessments [redacted]
- Complete urinalysis

- Administration of EO2401
- Infusion of nivolumab for Cohort 1, Cohorts 2a and 2b, and Cohort 3
- Infusion of bevacizumab only for Cohort 3
- Demographics, except height
- ECOG or Karnofsky performance status
- Physical examination
- Vital signs
- Assessment of AEs/toxicity
- Assessment of prior and concomitant medications
- Pregnancy test (if applicable)
- Blood collection [redacted]

7.4. Treatment-Boosting Period

Boosting injections of EO2401 will start 4 weeks after the fourth priming injection.

The visits during this period will occur until disease progression, death, or until the end of study. The treatment-boosting phase will be stopped in case of confirmed tumor progression, intolerable toxicity, death, or the study is terminated by the Sponsor.

7.4.1. [redacted]

The following assessments will be conducted:

- Clinical laboratory assessments [redacted]
- Complete urinalysis
- Infusion of nivolumab for Cohort 1, Cohorts 2a and 2b, and Cohort 3
- Infusion of bevacizumab only for Cohort 3
- Demographics, except height
- ECOG or Karnofsky performance status
- Physical examination
- Vital signs
- 12-lead ECG to be performed ± 4 days within scheduled time [redacted]
- [redacted]
  - [redacted]

- Tumor assessment: brain MRI to be performed +/- 4 days within scheduled time (for visits 5 and 9 only)
- NANO scale/questionnaire completion to assess neurological function (for Visits 5 and 9 only)
- Assessment of AEs/toxicity
- Assessment of prior and concomitant medications
- Blood collection [redacted]

7.4.2. [redacted]

The following assessments will be conducted:

- Clinical laboratory assessments [redacted]
- Complete urinalysis
- Administration of EO2401
- Infusion of nivolumab for Cohorts 1, Cohorts 2a and 2b, and Cohort 3
- Infusion of bevacizumab only for Cohort 3
- Demographics, except height
- ECOG or Karnofsky performance status
- Physical examination
- Vital signs
- Assessment of AEs/toxicity
- Assessment of prior and concomitant medications
- [redacted]
- [redacted]
- Pregnancy test (if applicable)
- Blood collection [redacted]

7.4.3. [redacted]

At Week n (every 2 weeks) the following will be conducted and all results will be documented:

- Clinical laboratory assessments [redacted] dipstick.

- [redacted]
- Complete urinalysis
- Infusion of nivolumab for Cohorts 1, 2, and 3 at dose of 3 mg/kg IV every 14 days according to SmPC[59] and US PI[60]
- Infusion of bevacizumab only for Cohort 3 at the dose of 10 mg/kg every 14 days as per standard treatment (US PI[69])
- Demographics, except height
- ECOG or Karnofsky performance status
- Complete physical examination of major body systems
- Vital signs
- Assessment of AEs/toxicity
- Assessment of prior and concomitant medications
- Tumor assessment: [redacted]
- NANO scale/questionnaire completion to assess neurological function to be performed [redacted].
- Pregnancy test (if applicable); [redacted]
- Blood collection [redacted]
- Survival status.

7.4.4. [redacted] End of Treatment or Early Discontinuation

At Week n + 2 weeks or in case of early termination, the following will be conducted and all results will be documented:

- Clinical laboratory assessments [redacted]

- [redacted]
- In case of treatment discontinuation, and after the first follow-up visit has been completed (including safety blood sampling) approximately 30 days after completion of last dose of study treatment, further safety blood sampling (including thyroid and adrenal glands function testing) in the context of the trial will be stopped if not clinically indicated (e.g. to follow-up study treatment toxicity) per the treating physician.
- Administration of EO2401 every 4 weeks until confirmed tumor progression, intolerable toxicity, death, or early termination of the study, where study treatment will be stopped and appropriate standard of care will be initiated by the Investigator
- Infusion of nivolumab for Cohorts 1, 2, and 3 at dose of 3 mg/kg IV every 14 days according to SmPC[59] and US PI[60]
- Infusion of bevacizumab only for Cohort 3 at the dose of 10 mg/kg every 14 days as per standard treatment (US PI[69])
- Demographics, except height
- ECOG or Karnofsky performance status
- Complete physical examination of major body systems
- Vital signs
- Assessment of AEs/toxicity
- Assessment of prior and concomitant medications
- Tumor assessment: [redacted]
- NANO scale/questionnaire completion to assess neurological function to be performed [redacted]
- [redacted]
- Pregnancy test (if applicable); [redacted]
- Blood collection [redacted]
- Survival status.

7.4.5. Follow-up Visits (After Progression or Permanent Treatment Discontinuation)

In case of treatment discontinuation, patients will complete monthly Follow-up Visits. The first Follow-up Visit will be completed approximately [redacted] after completion of last dose of study treatment. The clinical assessments performed below will be conducted. If the patient is unable to attend the site visit, safety follow-up via a telephone contact will be conducted and safety assessments recorded. The primary reason for premature study drug treatment discontinuation should be documented on the appropriate eCRF.

- If tests related to the EoT/Early discontinuation visit have not been done (see Section 7.4.4), they should be done during a follow-up visit.
- Physical examination (in case the patient comes to site visit)
- Vital signs (in case patient comes to site visit)
- Assessment of AEs/toxicity (see **Section 7.6.2.1**, **Section 7.6.3.6**, and **Section 7.6.3.7** regarding reporting periods for AEs and SAEs)
- Assessment of prior and concomitant medications
- Tumor assessment: [redacted]
- NANO scale/questionnaire completion to assess neurological function to be performed [redacted]
- A serum pregnancy test will be conducted [redacted] 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.
- Survival status.

7.5. Efficacy Assessments

The efficacy measurements used in the evaluations of the study endpoints are widely used and recognized as reliable, accurate, and conform to standard of care.

The assessment of PFS, ORR, DCR, DoR, and OS are considered secondary endpoints. The assessment of neurological function is considered an exploratory endpoint.

The efficacy assessments will be performed by MRI measurements every 8 weeks using the iRANO criteria (see Appendix 1).

7.5.1. Tumor and Response Evaluation

7.5.1.1. Acquisition Imaging

[redacted]

In this study, tumor response will be performed according to the Imaging Acquisition Guidelines (e.g., same imaging method, type of MRI, slice of thickness) to ensure the standardization of the technique, limit of potential bias, and ensure quality of the data. These guidelines will be provided in a separate document and delivered at each investigational site. The brain MRI might be read centrally following the Sponsor's decision. The brain MRI measurements will be conducted every 8 weeks[redacted], as per standard practices, using the criteria shown in Appendix 1 (iRANO criteria[77,78]) and will comprise of radiological and clinical assessments.

[redacted] the MRI scan must be performed [redacted]. If the next scheduled treatment administration is delayed (toxicity/other reasons), the disease assessment should be performed as initially planned (i.e., despite treatment delay, see Section 5.8.1.2).

An unscheduled MRI scan may be performed at the discretion of the Investigator to allow a decision on further study treatment administration for the patient.

For patients who discontinue treatment prematurely, disease assessment in the follow-up period should continue according to the original schedule and follow standard clinical practice until confirmed PD. If other anticancer treatment would be started before confirmed PD study related brain MRIs can be stopped

The same imaging method (MRI) as specified in the imaging guidelines must be used for a patient throughout the study and assessed by the same Investigator/radiologist during the study to the extent that is feasible.

Magnetic resonance imaging rules to be applied during the study:

- Anatomical coverage: Whole brain
- Recommendations
  - Tumor assessments will be performed using MRI of the brain with minimal field strength 0.5 Tesla systems or higher (3T)
  - The same MRI system should be used throughout the study
  - Brain coverage and acquisition parameters should remain consistent across all imaging visits for any subject.

Note: For this study, off protocol sequences not supported by RANO are:

- PET-CT scans
- MR-Spectroscopy

- Perfusion imaging.

7.5.1.2. Tumor Evaluation Using iRANO Criteria

Immunotherapeutic agents such as EO2316, EO2317, EO2318, and anti-PD(L)-1 therapy may produce antitumor effects by potentiating endogenous cancer-specific immune responses, which may manifest as initial worsening of enhancement and edema on MRI (i.e., PsPD). In addition, the response patterns seen with immunotherapeutics may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. For these reasons, the immune-related response criteria have endorsed continuation of study therapy beyond initial radiographic evidence of progression for clinically stable patients undergoing immune-based therapies.

A major advance of the RANO criteria to assess response in neuro-oncology over the previously used Macdonald criteria includes recognition of the prevalence of PsPD during the first 3 months following completion of radiation and daily temozolomide.[90] Specifically, RANO permits patients with such progressive MRI findings to continue temozolomide therapy for up to 3 months in order to avoid inaccurately classifying such patients as progressive. Furthermore, RANO permits patients with progressive radiographic findings at any time to continue current therapy pending follow-up imaging if the etiology of progressive imaging findings is unclear. Standard RANO may not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab.

Therefore, the following adaptations of the RANO criteria, as reflected in the iRANO criteria[77], will be used to assess response for patients treated on this study:

- Potential PsPD: If radiologic imaging shows initial PD, participants who are not experiencing significant clinical decline may be allowed to continue study treatment for up to 3 months. Patients who have radiographic evidence of further progression after up to 3 months, or who decline significantly at any time, will be classified as progressive with the date of disease progression back-dated to the first date that the participant met criteria for progression and such participants will be discontinued from study therapy. Although the kinetics of PsPD due to immune checkpoint blockade among GB patients are currently unknown, 3 months is a reasonable estimate based on: 1) the peak time for X-ray telescope/daily temozolomide-related PsPD is usually within 3 months of completion for GB patients[90] and; 2) 3 months is also the most common timeframe for PsPD observed among patients with advanced melanoma or other solid tumors treated with PD-1/PD(L)-1 immune checkpoint blockade to date.

Among patients on this study with initial radiographic PD, tumor assessment should be repeated regularly in order to confirm PD with the option of continuing treatment as described below while awaiting radiologic confirmation of progression. If repeat imaging shows a stabilization or reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued/resumed. If repeat imaging after up to 3 months confirms PD, then the date of disease progression will be the first date the participant met criteria for progression and participants will be discontinued from study therapy. Participants who have confirmed tumor (disease) progression will discontinue study medication and enter the follow-up/survival phase of the study. In determining whether or not the tumor burden has increased or decreased, Investigators should consider all target lesions as well as nontarget lesions.

In participants who have initial evidence of radiographic PD, it is at the discretion of the treating physician whether to continue a participant on-study treatment for up to 3 months pending confirmation of PD on follow-up imaging. This clinical judgment decision should be based on the participant's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Participants may receive study treatment while waiting for confirmation of PD if they are not experiencing significant clinical decline and if:

- The participant is believed to demonstrate clinical benefit from the study regimen as determined by the treating physician
- The participant is adequately tolerating study therapy.

When feasible, study therapy should not be discontinued until radiographic progression is confirmed. This allowance to continue treatment despite initial radiologic progression takes into account the observation that some participants can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. Participants that are exhibiting significant neurologic decline are not required to have repeat imaging for confirmation of PD.

7.5.2. Overall Survival

Overall survival will be defined as the time interval from the date of first study treatment administration to the date of death due to any cause.

Patients who are still alive at the time of analysis (clinical cutoff) and patients who are lost to follow-up will be censored at their last clinical assessment date.

7.5.3. Progression-free Survival

Progression-free survival is defined as the time interval from the date of first study treatment administration to the date of first occurrence of progression or death from any cause, whichever occurs first. The PFS will be assessed using the iRANO criteria (see Appendix 1). Patients without an event will be censored at the date of their last evaluable tumor assessment.

7.5.4. Objective Response Rate & Disease Control Rate

The ORR is defined as the proportion of patients with CR and PR according to the iRANO criteria. The DCR is defined as the proportion of patients with CR, PR, and Stable Disease (SD) according to the iRANO criteria.

7.5.5. Duration of Response

Duration of response will be defined as the time interval from the date of first occurrence of CR or PR to the date of first documentation of disease progression or death from any cause, whichever occurs first.

Duration of response will be calculated on responder patients. Only patients with a best overall response of CR or PR are considered responders.

7.5.6. Neurological Assessment of Neuro-Oncology Scale

The neurological function will be assessed using the NANO scale (see Appendix 2[75,76]). The NANO is a simple neurologic assessment by a means of a questionnaire conducted and reported by a trained health care professional who evaluates the patient in 9 domains: gait, strength, upper extremity ataxia, sensation, visual fields, facial strength, language, level of consciousness, and behavior. Each domain contains a score from 0 to 3 or 0 to 2, depending on the domain, with higher scores indicating worse neurologic function. The cumulative score can be compared over time to determine whether clinical status is stable, better, or worse as part of the response assessment.

[redacted]

7.6. Safety and Tolerability Assessments

Safety endpoints will be assessed by review of AE summaries, which will include TEAEs and SAEs, unless stated otherwise. Adverse events will be categorized by SOC and PT using the current MedDRA version and will be graded according to NCI-CTCAE v5.0.

7.6.1. Safety Plan

7.6.1.1. Management of Immune-related Adverse Reactions for Nivolumab and EO2401, and management of Dose Modifications for Adverse Reactions to Nivolumab

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 6 shows the recommended treatment management for different immune-related adverse reactions due to nivolumab; the same management principles should be utilized for EO2401 in case of immune-related adverse reactions (including potential immune cross-reactivity with normal tissue as described in **Section 1.3 Benefit-Risk Assessment**). For further guidance regarding treatment of immune-related adverse reactions during nivolumab treatment, e.g. corticosteroid dosing and hormone substitution therapy at immune-related endocrinopathies, refer to the SmPC[59] and US PI[60] for nivolumab.

Patients treated with nivolumab 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. Patients will be reviewed on a case-by-case basis, should they need to discontinue nivolumab due to toxicity. See Section 3.1 regarding general safety rules and guidance regarding continuation of individual components of the used combinations in this trial.

[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
	[redacted]	[redacted]
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[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]

[redacted]

Immune-related adverse reactions relating to nivolumab will be reported as an AE or an SAE, if the event fulfils the seriousness criteria (see Section 7.6.2.2) and will follow the applicable reporting procedures in Section 7.6.3.6 and Section 7.6.3.7.

Table 7B: Recommended Treatment Modifications for Nivolumab (in addition to Table 5A)

Adverse reaction	Severity	Action	Resume
Other Drug-Related AE **(not listed in Table 5A)**	Grade 2 non-skin AE, except fatigue	Delay dose	Dosing may resume when AE resolves to Grade ≤ 1 or baseline value.
	Grade 3 AE - First occurrence lasting ≤ 7 days	Delay dose	Dosing may resume when AE resolves to Grade ≤ 1 or baseline value.
	Grade 3 AE - First occurrence lasting > 7 days	Permanently discontinue	
	Recurrence of Grade 3 AE of any duration	Permanently discontinue	
	Grade 4 or Life-threatening adverse reaction	Permanently discontinue	
Other Lab abnormalities			
Other Drug-Related lab abnormality **(not listed in Table 5A)**	Grade 3	Delay dose	Exceptions: No delay required for: Grade 3 lymphopenia Permanent Discontinuation for: Grade 3 thrombocytopenia > 7 days or associated with bleeding.
	Grade 4	Permanently discontinue	Exceptions: The following events do not require discontinuation of study drug: • Grade 4 neutropenia ≤ 7 days • Grade 4 lymphopenia or leukopenia • Grade 4 isolated electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are responding to supplementation/appropriate management within 72 hours of their onset
Infusion Reactions (manifested by fever, chills, rigors, headache, rash, pruritus, arthralgia, hypotension, hypertension, bronchospasm, or other allergic-like reactions.)			
Hypersensitivity reaction or infusion reaction	Grade 3 or 4	Permanently discontinue	Refer to **Section 7.6.1.2** and **Appendix 7** for management of infusion reactions

Side effects related to nivolumab are presented in Section 1.2.1.3.1.1. See also details in the SmPC[59] and US PI[60] for nivolumab, ESMO,[3, 92] and Food and Drug Administration guidelines.[93]

In case of nivolumab treatment discontinuation due to related AE, consult **Section 3.1** regarding the possibility to continue other treatment components.

7.6.1.2. Management of Infusion Reactions to Nivolumab

Severe infusion reactions have been reported in clinical trials using nivolumab. In case of a severe or life-threatening infusion reaction, the nivolumab infusion must be discontinued and appropriate medical therapy must be administered. Patients with mild or moderate infusion reaction may receive nivolumab with close monitoring and use of premedication according to local treatment guidelines for prophylaxis of infusion reactions.

Hypersensitivity

Nivolumab should be permanently discontinued in patients exhibiting hypersensitivity/allergic 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.

Patients may be at risk of developing infusion reactions to nivolumab. Close observation of the patient during and following the administration of nivolumab is recommended, as expected for any infusion of a therapeutic humanized monoclonal antibody. If an infusion reaction occurs, the infusion should be discontinued and appropriate medical therapies should be administered. A systematic premedication is not warranted.[60]

See also details in the SmPC[59] and US PI[60] for nivolumab, ESMO,[3] and Food and Drug Administration guidelines,[94] and please refer to Appendix 7.

7.6.1.3. Management of Dose Modifications for Adverse Reactions to Bevacizumab

Details of dose modifications for adverse reactions to bevacizumab are provided in Table 8.

Table 8: Dose Modifications for Adverse Reactions to Bevacizumab

Adverse Reaction	Severity	Dose Modification
Gastrointestinal Perforation and fistulae (see Warnings and Precautions [5.1])	• Gastrointestinal perforation, any grade • Tracheoesophageal fistulae, any grade • Fistulae, Grade 4 • Fistulae formation involving any internal organ	Discontinue bevacizumab

Adverse Reaction	Severity	Dose Modification
Wound Healing Complications (see Warnings and Precautions [5.2])	• Wound healing complications requiring medical intervention • Necrotizing fasciitis	Discontinue bevacizumab
Hemorrhage (see Warnings and Precautions [5.3])	• Grade 3 or 4	Discontinue bevacizumab
	• Recent history of hemoptysis of ½ teaspoon (2.5 mL) or more	Withhold bevacizumab
Thromboembolic Events (see Warnings and Precautions [5.4, 5.5])	• Arterial thromboembolism, severe	Discontinue bevacizumab
	• Venous thromboembolism, Grade 4	Discontinue bevacizumab
Hypertension (see Warnings and Precautions [5.6])	• Hypertensive crisis • Hypertensive encephalopathy	Discontinue bevacizumab
	• Hypertension, severe	Withhold bevacizumab if not controlled with medical management; resume once controlled
Posterior Reversible Encephalopathy Syndrome (see Warnings and Precautions [5.7])	• Any	Discontinue bevacizumab
Renal Toxicity and Proteinuria (see Warnings and Precautions [5.8])	• 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 (see Warnings and Precautions [5.10])	• Severe infusions reaction	Discontinue bevacizumab
	• Clinically significant	Interrupt infusion; resume at a decreased rate of infusion after symptoms resolve
	• Mild, clinically insignificant	Decrease infusion rate
Congestive Heart Failure (see Warnings and Precautions [5.12])	• Any	Discontinue bevacizumab

Reference source: US PI[69]

7.6.2. Safety Parameters and Definitions

7.6.2.1. Adverse Events

Adverse events will be monitored throughout the study. Adverse events will be collected from the time informed consent is signed and continue until 30 days after the last study treatment is administered, until all serious or study treatment-related toxicities have resolved or are determined to be "chronic" or "stable," completion of the patient's participation, study termination (e.g., patient lost to follow-up), or the Investigator or designee and Sponsor agree that follow-up is no longer necessary, whichever occurs first.

In addition, for consistency with nivolumab clinical trial protocols as advised by Bristol-Myers Squibb (marketing authorization holder for nivolumab), collect 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. The most conservative approach (longest follow-up) emanating from the two principles (30 days and specifics per above paragraph versus 100 days following discontinuation of nivolumab treatment) should be followed for nivolumab.

For the purpose of this study, the terms toxicity and AE are used interchangeably.

The Investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the patient to discontinue the study drug or study (see Section 4.3.3).

Definition

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related. This includes the following:

- Any clinically significant worsening of a pre-existing condition
- **Note:** Emergence of a new pathogen associated with a clinical event during therapy at a site other than the initial site of infection will be considered an AE
- Any recurrence of a pre-existing condition
- An AE occurring from overdose of a Sponsor study drug whether accidental or intentional (i.e., a dose higher than that prescribed by a health care professional for clinical reasons)
- An AE occurring from abuse of a Sponsor study drug (i.e., use for nonclinical reasons)
- An AE that has been associated with the discontinuation of the use of a Sponsor study drug.

Note: A procedure is not an AE, but the reason for a procedure may be an AE.

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.

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: (1) 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 (2) 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 Follow-up Visit.

Abnormal laboratory and other abnormal investigational findings (i.e., physical exam) should not be reported as AEs, unless they are associated with clinical signs and symptoms, lead to

treatment discontinuation, or are otherwise considered clinically relevant by the Investigator. In cases of surgical or diagnostic procedures, the condition/illness leading to such a procedure is considered as the AE rather than the procedure itself.

In a case of fatality, the cause of death is considered as the SAE, and the death is considered as its outcome, and the end date of the SAE is the date of death.

Pre-existing medical conditions/diseases/symptoms that worsen after informed consent are signed are considered AEs regardless of causality with study treatment. Abnormal laboratory values or test results constitute AEs only if they induce clinical signs and symptoms, are considered clinically significant, or require therapy.

Information about all AEs, whether volunteered by the patient, discovered by the Investigator or designee questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded, as appropriate.

All AEs will be treated appropriately. Such treatment may include changes in study treatment as listed in the dose modification section of the protocol (Section 5.8.1).

An AE or SAE will be considered treatment-emergent if they occur any time after the first dose of study treatment.

7.6.2.2. Serious Adverse Event

An SAE is any AE occurring at any dose that meets one or more of the following criteria:

- Results in death
- Is life-threatening (see below)
- Requires patient hospitalization or prolongation of an existing hospitalization (see below)
- Results in a persistent or significant disability or incapacity (see below)
- Results in a congenital anomaly or birth defect
- Results in an important medical event (see below).

Additionally, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered as SAEs when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not require hospitalization, or development of drug dependency or drug abuse.

A ***life-threatening adverse event*** is any AE that places the patient at immediate risk of death from the event as it occurred. A life-threatening event does not include an event that might have caused death had it occurred in a more severe form but that did not create an immediate risk of death as it actually occurred. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though drug-induced hepatitis of a more severe nature can be fatal.

Hospitalization or prolongation of a hospitalization is a criterion for considering an AE to be serious. In the absence of an AE, the participating Investigator should not report hospitalization or prolongation of hospitalization. This is the case in the following situations:

- Hospitalization or prolongation of hospitalization is needed for a procedure required by the protocol. Day or night survey visits for biopsy or surgery required by the protocol are not considered serious.
- Hospitalization or prolongation of hospitalization is part of a routine procedure followed by the study center (e.g., stent removal after surgery). This should be recorded in the study file.
- Hospitalization for survey visits or annual physicals fall in the same category.

In addition, a hospitalization planned before the start of the study for a pre-existing condition that has not worsened does not constitute an SAE (e.g., elective hospitalization for a total knee replacement due to a pre-existing condition of osteoarthritis of the knee that has not worsened during the study). Hospitalization is to be considered only as an overnight admission.

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).

If there is any doubt as to whether a case constitutes an AE or SAE based on the information available, the case should be treated as an SAE.

Medical and scientific judgment should be exercised in deciding whether a case is serious in those situations where important medical events may not be immediately life-threatening or result in death, hospitalization, disability, or incapacity. These include events that may jeopardize the patient or may require medical intervention to prevent one or more outcomes listed in the definition of serious. Such events should usually be considered as serious.

Toxicities that fall within the SAE definitions listed above must be reported as an SAE, regardless if they are felt to be treatment-related or not. Toxicities unrelated to treatment that do NOT fall within the SAE definitions above must be documented as AEs in the patient's source documents and eCRF.

7.6.2.3. Suspected Unexpected Serious Adverse Reactions

Suspected unexpected serious adverse reactions (SUSARs) are serious events that are not listed in the EO2401 Reference Safety Information section of the Investigator Brochure. Suspected adverse reaction means any AE for which there is a reasonable possibility that the 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 drug and the AE. As this is an FIH trial, all related SAEs are considered SUSARs.

7.6.2.4. Adverse Drug Reactions

All noxious and unintended responses to an IMP (i.e., where a causal relationship between an IMP and an AE is at least a reasonable possibility) related to any dose should be considered adverse drug reactions.

For marketed medicinal products, a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved IMP).

7.6.3. Methods and Timings for Capturing and Assessing Safety Parameters

7.6.3.1. Follow-up of Adverse Events/Serious Adverse Events

Adverse events/SAEs will be followed until resolution or stabilization of the event, completion of the patient's participation, or study termination, or the Investigator or designee and Sponsor agree that follow-up is no longer necessary, whichever occurs first.

Assessments should be made at each visit (or more frequently, if necessary) of any changes in severity, the relationship to the study drug, the intervention required to treat it, and the outcome.

Common, uncommon, and rare side effects known for nivolumab are provided in the SmPC.[62] All AEs will be immediately recorded in the patient's source documents.

7.6.3.2. Assessment of Severity of Adverse Events

The severity grade will be based on the NCI-CTCAE v5.0. An electronic copy of the NCI-CTCAE v5.0 can be downloaded from https://ctep.cancer.gov. As soon as the site enters a grade ≥3 AE, the Medical Monitor will be immediately informed through a specific alert programmed in the e-CRF**.**

7.6.3.3. Assessment of Causality of Adverse Events

The Investigator must assess and document causal relationship between an SAE and the study drug on the basis of his/her clinical judgment using the following definitions. The causality assessment must be made based on the available information and can be updated as new information becomes available.

Is there a "reasonable possibility" that EO2401, or when applicable the combination of EO2401 + nivolumab, or EO2401 + nivolumab + bevacizumab caused the AE (meaning there is evidence to suggest a causal relationship the administered treatment and the AE)?

- Yes
- No.

Could the AE be explained by underlying disease or other drugs or chemicals?

- Yes
- No.

7.6.3.4. Action Taken for Adverse Events

The Investigator or designee will record the action taken for the AE in the eCRF. Actions taken will include:

- **Dose not changed**: The medication schedule was not changed
- **Drug interrupted**: The medication schedule was modified by temporarily terminating the prescribed regimen of medication
- **Drug withdrawn**: The medication schedule was modified through termination of the prescribed regimen of medication
- **Not applicable**
- **Unknown**.

7.6.3.5. Adverse Event Outcome

- **Recovered/resolved**: The patient fully recovered from the AE with no residual effect observed
- **Recovered/resolved with sequelae**: The residual effects (e.g., symptoms or pathology) of the AE are still present and observable
- **Not recovered/not resolved**: The AE itself is still present and observable
- **Fatal**: The patient dies as a result of the AE
- **Unknown**: Outcome of the AE is unknown.

7.6.3.6. Reporting Adverse Events

At each visit, the Investigator, or delegate, will determine whether or not any AEs have occurred. Nonleading questions such as "How are you feeling today?" or "Have you had any health concerns since your last visit?" should be used to elicit the patient to report any possible AEs. If any AEs have occurred, they will be recorded in the AE section of the eCRF and in the patient's source documents. If known, the diagnosis should be recorded in preference to listing the individual signs and symptoms.

Furthermore, the Investigator is responsible for ensuring that any sub-Investigator promptly brings AEs to the attention of the Investigator. If applicable, as per national regulation, the Investigator is also responsible for informing the site's IEC/IRB of all SAEs (per IEC/IRB requirements).

Adverse event reporting begins from the time informed consent is signed and ends 30 days after the last treatment administration or until all serious or study treatment-related toxicities have resolved or are determined to be "chronic" or "stable," completion of the patient's participation, study termination (e.g., patient is lost to follow-up), or the Investigator or designee and Sponsor agree that follow-up is no longer necessary, whichever occurs first.

In addition, for consistency with nivolumab clinical trial protocols as advised by Bristol-Myers Squibb (marketing authorization holder for nivolumab), collect 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. The most conservative approach (longest follow-up) emanating from the two principles (30 days and specifics per above paragraph versus 100 days following discontinuation of nivolumab treatment) should be followed for nivolumab.

[redacted]

7.6.3.7. Reporting Serious Adverse Events

All SAEs, occurring after the signing of the ICF until 30 days after the last study treatment and regardless of study drug relationship, must be entered into the clinical database as an AE (with appropriate indication that the AE represents an SAE) and must be submitted according to the below process (paper SAE form) to the Enterome/[redacted] within 24 hours of obtaining knowledge of the event. The Investigator should enter all available information requested on the SAE Form.

[redacted]

The SAE Form will 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 an AE page has been updated in the eCRF indicating an SAE by the site, an alert from the clinical database will be initiated and sent to the Enterome/[redacted]. If the paper SAE Form has not been received by the Enterome/[redacted] form the site within 24 hours after such an alert the site will be contacted and queried for the SAE Form.

The Enterome/[redacted] will generate SAE queries requesting incomplete, implausible, or missing information. It is the Investigator's responsibility to be diligent in providing the answer as soon as it is available by entry of the applicable corrections in the eCRF and submission of the follow-up SAE report to the Enterome/[redacted].

The paper SAE Form will be provided to the Investigators prior to start of the study.

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.

Initial reports of SAEs should never be left on telephone voicemails. Please always fax or email the SAE Forms and follow with a telephone call if needed.

Note, all SAE Forms must be completed in English.

Serious AEs to be reported to the [redacted] Safety Group:

Facsimile: [redacted]

E-mail: [redacted]

In case of urgent questions regarding SAE reporting please call:

Enterome Medical Monitor [redacted]

[redacted] [redacted]

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

7.6.3.8. Procedures for Recording Adverse Events

7.6.3.8.1. Infusion-related Reactions

Reactions temporally associated with infusions

Adverse events that occur during or within 24 hours after study treatment administration should be captured as individual signs and symptoms on the AE eCRF page rather than an overall diagnosis (e.g., record dyspnea and hypotension as separate events rather than a diagnosis of infusion-related reaction).

7.6.3.8.2. Diagnosis versus Signs and Symptoms

For AEs other than infusion-related reactions (see Section 7.6.3.8.1), a diagnosis (if known) should be recorded on the AE eCRF page rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded on the AE eCRF page. If a diagnosis is subsequently established, all previously reported AEs based on signs and symptoms should be nullified and replaced by 1 AE report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

7.6.3.8.3. Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between clinical visits. Such events should only be recorded once on the AE eCRF page. The initial severity (CTCAE grade) of the event will be recorded at the time the event is first reported. If a persistent AE becomes more severe, the most extreme severity should also be recorded on the AE eCRF page. If the event becomes serious, it should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning that the event became serious; see Section 7.6.3.7 for reporting instructions). The AE eCRF page should be updated by changing the event from "nonserious" to "serious," providing the date that the event became serious, and completing all data fields related to SAEs.

A recurrent AE is one that resolves between patient evaluation timepoints and subsequently recurs. Each recurrence of an AE should be recorded separately on the AE eCRF page.

7.6.3.8.4. Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an AE. A laboratory test result should be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., treatment interruption or treatment discontinuation)
- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy
- Is clinically significant in the Investigator's judgment.

It is the Investigator's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase 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."

Observations of the same clinically significant laboratory abnormality from visit to visit should only be recorded once on the AE eCRF page (see Section 7.6.3.8.3 for details on recording persistent AEs).

7.6.3.8.5. Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an AE. A vital sign result should be reported as an AE if it meets any of the following criteria:

- Accompanied by clinical symptoms
- Results in a change in study treatment (e.g., treatment interruption or treatment discontinuation)
- Results in a medical intervention or a change in concomitant therapy
- Clinically significant in the Investigator's judgment.

It is the Investigator's responsibility to review all vital sign findings. Medical and scientific judgment should be exercised in deciding whether an isolated vital sign abnormality should be classified as an AE.

Vital sign measurements outside the normal ranges will be assessed as "abnormal, not clinically significant" or "abnormal, clinically significant" by the Investigator. In the latter case, the

abnormal vital sign measurement will be reported as an AE and further investigated as clinically indicated.

7.6.3.8.6. Abnormal Liver Function Values

The finding of an elevated ALT or AST (> 3 ×ULN) in combination with either an elevated total bilirubin (> 2 ×ULN) or clinical jaundice in the absence of cholestasis or other causes of hyperbilirubinemia is considered to be an indicator of severe liver injury. Therefore, Investigators must report as an AE the occurrence of either of the following:

- Treatment-emergent ALT or AST > 3 ×ULN in combination with total bilirubin > 2 ×ULN
- Treatment-emergent ALT or AST > 3 ×ULN in combination with clinical jaundice.

The most appropriate diagnosis or (if a diagnosis cannot be established) the abnormal laboratory values should be recorded on the AE eCRF page (see Section 7.6.3.6) and in addition reported to the Sponsor via email from the site. Potential events of drug-induced liver injury (DILI) will be reported as SAEs if seriousness criteria are fulfilled (see Section 7.6.3.7). All patients with potential DILI will be closely followed until abnormalities return to normal or baseline or until all attempts to determine resolution of the event are exhausted.

7.6.3.8.7. Death

Deaths that occur during the protocol-specified AE reporting period that are attributed by the Investigator solely to disease progression should be recorded only on the EoT eCRF page. All other on-study deaths, regardless of relationship to study drug, must be recorded on the AE eCRF page and immediately reported to the Sponsor. The IDMC will Monitor the frequency of deaths from all causes.

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the AE eCRF page. Generally, only one such event should be reported. The term “sudden death” should only be used for the occurrence of an abrupt and unexpected death due to presumed cardiac causes in a patient with or without pre-existing heart disease, within 1 hour of the onset of acute symptoms or, in the case of an unwitnessed death, within 24 hours after the patient was last seen alive and stable.

If the cause of death is unknown and cannot be ascertained at the time of reporting, “unexplained death” should be recorded on the AE eCRF page. If the cause of death later becomes available (e.g., after autopsy), “unexplained death” should be replaced by the established cause of death.

Since death is a seriousness criterion (see Section 7.6.2.2), the events leading to death should be reported to the CRO following the instructions in Section 7.6.3.7.

7.6.3.8.8. Pre-existing Medical Conditions

A pre-existing medical condition is one that is present at the Screening Visit for this study. Such conditions should be recorded on the Medical History eCRF page.

A pre-existing medical condition should be recorded as an AE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the AE

eCRF page, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors (e.g., "more frequent headaches").

7.6.3.8.9. Disease Progression

Events that are clearly associated with progression of the underlying disease should not be recorded as an AE. These events will be captured as efficacy assessment data only. In most cases, the expected pattern of progression will be based on protocol-defined response criteria (e.g., iRANO). In rare cases, the determination of clinical progression will be based on symptomatic deterioration. However, every effort should be made to document progression using objective criteria.

Hospitalization due solely to progression of the underlying cancer should not be reported as an SAE.

Deaths that are attributed by the Investigator solely to progression of disease should only be reported on "End of Study" eCRF page and not as an SAE. All other on-study events that led to death during the protocol-specified AE reporting period, regardless of relationship to study drug, must be recorded on the "AE" eCRF page as SAEs and immediately reported to the Sponsor, as specified in Section 7.6.3.7, reporting of SAEs.

7.6.3.8.10. 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 7.6.3.7 for reporting instructions).

7.6.4. Investigator's Notification of Adverse Events to the Sponsor

All Investigators will be thoroughly instructed and trained on all relevant aspects of the Investigator's reporting obligations for SAEs. This information, including all relevant contact details, is summarized in the investigational site file. This information will be updated as needed. Serious AEs occurring during the study must immediately (within 24 hours of Investigator awareness) be reported per Section 7.6.3.7.

Serious AEs occurring after the protocol-defined observation period will be processed by the Sponsor according to applicable regulations (see Section 7.6.6).

7.6.5. Expedited Reporting of Adverse Events to Regulatory Agencies/Authorities

7.6.5.1. Notification to the Ethics Committee/Institutional Review Board

Notification to the IEC/IRB about all relevant events (e.g., SAEs/SUSARs) will be performed by the Sponsor or Sponsor's designee and/or by the Investigator according to all applicable rules/regulations.

7.6.5.2. Notification to the Authorities

The processing and reporting of all relevant events (e.g., SAEs) to the authorities will be done by the Sponsor or Sponsor's designee and/or by the Investigator according to all applicable rules/regulations.

7.6.5.3. Sponsor's Notification to the Investigational Site

The Sponsor or designee will inform all investigational sites about reported relevant events according to applicable regulations. The Sponsor or Sponsor's designee will send the events to a site once the site initiation visit has occurred and will stop sending to the site once the last patient has completed their EoT Visit.

7.6.6. Post-study Adverse Events

The Sponsor should be notified if the Investigator becomes aware of any SAEs that occurs after the end of the AE reporting period (see Section 7.6.2.1), if the event is believed to be related to prior study treatment.

The Investigator should report these post-study events to the Sponsor or its designee, either by faxing or by scanning and emailing the SAE Reporting Form using the fax number or email address provided to Investigators.

7.6.7. Independent Data Monitoring Committee

The IDMC will serve as a monitory advisory group for the study. The primary role of the IDMC will be to examine the safety and tolerability of study participates throughout the duration of the study. The IDMC will be created to further protect the rights, safety, and well-being of patients who will be participating in the trial by monitoring their progress and results. [redacted]

The major tasks for the IDMC are outlined in relation to the description of the design of the trial (see Section 3.1).

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

[redacted]

The IDMC comprises qualified, international experts [redacted] who are not Investigators in the study and not otherwise directly associated with the Sponsor. The IDMC will be described in detail in the IDMC Charter.

7.6.8. Pregnancy

Although not considered an AE, it is the responsibility of the Investigator or their designee to report any pregnancy in a patient or the patient's sexual partner that occurs during the study. All patients who become pregnant must immediately discontinue the study drug and be withdrawn from the study. The patient will be followed to completion/termination of the pregnancy. This information is important for both drug safety and public health concerns. If a patient is found to be pregnant after the study treatment was administered, the Investigator should report this to the CRO and Sponsor immediately and document the pregnancy on the Pregnancy Form in the eCRF.

The Investigator must make every effort to follow the patient until completion of pregnancy and provide the corresponding information by the mean of a SAE/Pregnancy form within 2 weeks from awareness (same reporting procedures apply as for SAE reporting, see Section 7.6.3.7). The Enterome/[redacted] will periodically request the investigator for targeted follow-up information using specific pregnancy follow-up forms. If the events during pregnancy and/or outcome of pregnancy (i.e., complications regarding mother/baby) meet the criteria for classification of an SAE (see Section 7.6.2.2), the Investigator must follow the procedures for reporting SAEs outlined in Section 7.6.3.7.

7.7. Clinical Laboratory Evaluations

The timepoints at which these samples will be collected are shown in the Schedule of Assessments (Table 1). [redacted]

The following clinical laboratory assessments (not exhaustive list) will be performed:

- **Hematology panel**: [redacted]
- **Blood chemistry/electrolytes**: [redacted]
- **Renal function**: [redacted]
- **Hormonal function**: [redacted]
- **Coagulation panel**: [redacted]
- **Urinalysis** [redacted].

A full list of laboratory parameters is provided in Appendix 5.

Any clinically significant abnormal laboratory value should be immediately rechecked, whenever possible, for confirmation before making any decision for the concerned patient. It should be graded by NCI-CTCAE criteria v5.0 and documented as an AE/SAE PP requirement. For procedures on reporting abnormal laboratory values and liver function values, refer to Section 7.6.3.8.4 and Section 7.6.3.8.6, respectively.

[redacted]

A serum pregnancy test will be conducted [redacted] This is not required for females who have undergone and have documentation of a hysterectomy.

Pregnancy tests will be performed as clinically indicated, or as required by local regulations, thereafter.

Pregnancy tests may be performed at other visits at the discretion of the Investigator.

Postmenopausal status will be confirmed [redacted]

7.7.1. [redacted]

[redacted]

[redacted]

7.7.2. Immune Response Evaluation

Cell-mediated cytotoxicity is a key element in the proposed mechanism of action of the peptides composing EO2401. [redacted] Selection of an ex-vivo monitoring technique that provides the best measure of immune reactivity is important in determining potential correlations between clinical and immunologic responsiveness to specific immunotherapy.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

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

7.7.3. [redacted]

[redacted]

[redacted]

7.8. Vital Signs, Physical Examination, and Other Safety Evaluations

7.8.1. Vital Signs and ECOG or Karnofsky Performance Status

Measurement of vital signs will include an assessment of HR, systolic and diastolic BP, temperature, and respiratory rate.

Blood pressure and HR will be measured 5 minutes in a sitting or semi-recumbent position by means of oscillometry, using an automatic BP measuring device. It is recommended that the patient rests for 5 minutes before BP is measured. [redacted]

Vital sign measurements outside the normal ranges will be assessed as "abnormal, not clinically significant" or "abnormal, clinically significant" by the Investigator. In the latter case, the abnormal vital sign measurement will be reported as an AE and further investigated as clinically indicated (see Section 7.6.3.8.5).

The ECOG or Karnofsky performance status will be collected throughout the study (see Appendix 3).

7.8.2. 12-Lead Electrocardiogram

A single standard 12-lead ECG will be conducted as a triplicate trace at Screening and should be performed within 2 months before Screening. These 3 ECG will be performed within a 5-minute interval between each of these 3 ECGs. Additional ECGs may be performed during the study as indicated in the Schedule of Assessments (Table 1) [redacted] ECG will be performed in case of medical needs. Electrocardiogram performed at different timepoints and at early discontinuation of the study will be conducted prior treatment (EO2401 and/or nivolumab bevacizumab, as appropriate) administration and at the discretion of the Investigator. New or worsened abnormalities should be recorded as AEs on the eCRF.

Patients will be monitored by standard 12-lead ECG conducted according to local practice.

7.8.3. Physical Examination

Physical examination will include an examination of major body systems. [redacted]

Height will be measured once during Screening. Body weight will be measured throughout the study to EoT or early discontinuation of the study at every clinic visit, if possible. Vital signs may be repeated for clinically significant abnormal findings at the discretion of the Investigator.

Abnormal findings on physical exam will be documented in the source documentation and eCRF. New or worsened abnormalities should be recorded as AEs, if applicable.

8. SAMPLE SIZE AND DATA ANALYSES

8.1. Determination of Sample Size

As this is the FIH trial of EO2401, there is no formal statistical analysis planned. Individual patient data and summary tables of data will be presented. The design builds on a 3-by-3 approach for Cohort 1 [redacted] and then a goal of having a sufficient, but not unnecessarily high, number of evaluable patients for safety data, immunogenicity, and early efficacy assessments in each of the cohorts (for safety and feasibility only, assessments less patients are accepted); the actual and planned patient numbers per cohort are:

- Cohort 1a (sEN treatment of patients with measurable disease): 3 patients in the safety lead-in part, plus 18 added patients by the global amendment 2 plus re-distribution of patients from Cohort 2b, also including extended patient management measures = total of 21 patients (recruitment finalized before global amendment 3)

- Cohort 2a (EN treatment of patients with measurable disease): 23 patients before adding 15 further patients by the global amendment 2 plus re-distribution of patients from Cohort 2b, also including extended patient management measures = total of 38 patients (recruitment finalized before global amendment 3)

- Cohort 2b (EN adjuvant treatment, no measurable disease): lowered from 15 patients to 6 patients by re-distribution to Cohorts 1a and 2a (recruitment finalized before global amendment 3), by the global amendment 2 also including extended patient management measures

- Cohort 2c (EN neoadjuvant treatment - surgery - adjuvant treatment); cohort added by the global amendment 2 to evaluate safety and feasibility of the approach in 6 patients, also including extended patient management measures

- Cohort 3 (ENB treatment of patients with measurable disease): by global amendment 3 target recruitment increase from 10 to 26 patients, by the global amendment 2 also including extended patient management measures

- *Total of approximately 100 patients; sEN = "slow" EO2401/nivolumab (delayed N for 4 weeks); EN = EO2401/nivolumab (combination treatment from start); ENB = EO2401/nivolumab/bevacizumab (bevacizumab full dose according to US label, and only recruiting in the US)*

[redacted]

Data obtained from this study will be used to power future studies, and for this purpose the three key cohorts, i.e. Cohorts 1a/2a/3, all include more than 20 patients.

Given the exploratory nature of this Phase Ib/IIa study, the sample size is not based on power calculation. The number of patients to be included will provide sufficient safety and tolerability, and immunogenicity data, as well as preliminary efficacy data without exposing too many patients.

8.2. Analysis Populations

The following analysis populations will be included for this study:

The **All Patient Population** will consist of any patient who signed informed consent including Screen failures.

Full Analysis Set will consist of any patients who received at least 1 dose of study drug (EO2401 alone or EO2401 in combination with nivolumab, or nivolumab/bevacizumab) for whom no important protocol deviations occurred. Patients who are not considered evaluable for this population will not be replaced.

Safety Population will consist of any patients who received at least 1 dose of study drug (EO2401 alone or EO2401 in combination with nivolumab or nivolumab/bevacizumab). Patients who are not considered evaluable for this population will be replaced.

Per-Protocol Population will consist of any patients who received [redacted] study drug (EO2401 alone or EO2401 in combination with nivolumab or nivolumab/bevacizumab) for whom no important protocol deviations occurred and have at least 1 evaluable post-Screening tumor assessment. Patients who are not considered evaluable for this population will not be replaced, and be included in the FAS.

Efficacy will be analyzed using both the FAS and PP Populations. Safety will be analyzed using the Safety Population.

8.3. General Considerations

The following is an overview of the statistical analysis methods to be used in this study. Details on the statistical analyses will be given in the Statistical Analysis Plan.

A data review meeting will be held before database lock. Protocol deviations will be reviewed during the data review meeting. Furthermore, assignment of patients to the analysis set will be performed.

All data collected in this study will be documented with the help of patient data listings and summary tables and figures. Data listings will be provided for the different analysis populations as specified in the Statistical Analysis Plan (SAP). Summary statistics and statistical analysis, where required, will be performed for patients included in the relevant analysis populations (Safety/FAS/PP). Unless stated otherwise, descriptive summary statistics will include frequency counts and percentages for categorical variables and number of observations, mean, standard deviation, median, minimum, and maximum and the first and third quartiles for continuous variables.

Missing values will not be imputed.

[redacted]

In addition, an update of OS analysis and listings of efficacy and safety parameters for patients who are still alive in the study after the interim analysis will be performed on a regular basis (every 3 to 6 months, as appropriate).

The timing of the final analysis will be defined in the Statistical Analysis Plan and possibly amended in relation to the outcome of interim analysis 1 and 2.

The final analysis will be done when the last patient completes the study treatment, all assessments for disease response, and monthly Follow-up Visits (see Sections 7.4.4 and 7.4.5). The first Follow-up Visit will be completed approximately 30 days after termination of study treatment.

[redacted]

It is planned for all patients to be followed for survival for 24 months after last patient enrollment, if possible, via hospital records or other registers.

Statistical analysis will be performed using SAS version 9.4 or later.

8.3.1. Repeat and Unscheduled Readings

Repeat assessments occur when the original result requires confirmation. Repeat assessments are handled and documented per the SAP. Prior to first dosing, all assessments taken in addition to the original assessment are defined as pre-dose repeats. Post-dose repeat assessment is defined as assessments collected within 15 minutes of the actual time of the original assessment.

8.3.2. Demographic and Other Baseline Characteristics

8.3.2.1. Patient Disposition

The number and percentage of patients screened and treated will be presented by cohort and listed per the SAP. The reasons for patients discontinued from treatment will be summarized by cohort. In addition, the number of patients screened and included in each analysis population will be displayed by center.

8.3.2.2. Patient and Disease Characteristics

Descriptive summary statistics and frequency counts of demographic (age, sex, race, ethnicity, body weight, and height) and disease characteristics will be presented by cohort and overall per the SAP. Demographic and disease variables will also be listed.

8.3.3. Medical and Surgical History

Medical and surgical history includes relevant history other than GB. The information will be coded using the current version of MedDRA. Coding terms and Investigator terms will be given in the listings.

8.3.4. Prior and Concomitant Medications

All medications will be coded using the WHO Drug Dictionary and Anatomical Therapeutic Chemical Classification system with version numbers as defined in the SAP. Coding terms and Investigator terms will be given in the listings.

Prior medications will be defined as none study medication with a stop date prior to the first dose of study treatment.

8.3.5. Tissue Sample Assessment

Descriptive summary statistics and frequency counts of IL-13Rα2/BIRC5 (survivin)/FOXM1 and PD(L)-1 expression using IHC technique will be presented by cohort and overall per the SAP.

8.4. Efficacy Analysis

The efficacy assessments in this study are considered secondary and exploratory [redacted] The definition of these efficacy endpoints is provided in Section 2.2.3. Routine tumor follow-up including tumor staging will be evaluated using descriptive statistics and frequency tables as appropriate.

The efficacy assessments will be performed by MRI measurements every 8 weeks using the iRANO criteria (see Appendix 1).

8.4.1. Overall Survival

The OS will be summarized descriptively overall and per cohort using the Kaplan-Meier method. The median survival time will be presented with its associated 95% confidence interval (CI). The results will also be presented graphically in Kaplan-Meier plot. Survival rate assessments will be made at timepoints defined in the Statistical Analysis Plan.

8.4.2. Progression-free Survival

The PFS will be summarized descriptively overall and per cohort using the Kaplan-Meier method. The median PFS time will be presented with its associated 95% CI. The results will also be presented graphically in a Kaplan-Meier plot.

8.4.3. Objective Response Rate & Disease Control Rate

The ORR will be estimated overall and per cohort and provided with its 95% CI. The DCR will be estimated overall and per cohort and provided with its 95% CI.

8.4.4. Duration of Response

Response duration will be calculated on responder patients (CR and PR) only and will be displayed as time to event parameter using the Kaplan-Meier analysis.

8.4.5. Neurological Assessment of Neuro-Oncology Scale

The NANO score will be summarized descriptively as outlined in the SAP.

8.5. Safety Analysis

Safety variables include incidence of AEs (or TEAEs), SAEs, laboratory test results, vital signs, ECOG performance, and ECG results. All safety analyses will be based on the Safety Population. No formal statistical analysis of the safety data will be performed.

Summary tables will be provided for all TEAEs by cohort. The incidence of AEs, related AEs/TEAEs, SAEs, and AEs leading to discontinuation of the study treatment will be presented by the current MedDRA version, SOC and PT. In addition, the incidence of AEs by severity will be presented by SOC and PT. Listings will be produced for all AEs (TEAEs and non-treatment-emergent). Listings will be provided for those patients who experience an SAE, including death, or who experience an AE associated with early withdrawal from the study or from study drug treatment.

The TEAE summary tables will include counts of patients. Therefore, if a patient experiences more than 1 episode of a particular AE, the patient will be counted only once for that event. If a patient has more than 1 TEAE that is coded to the same PT, the patient will be counted only once for that PT. Similarly, if a patient has more than 1 TEAE within an SOC, the patient will be counted only once in that SOC.

Clinical laboratory test variables will be summarized by cohort and visit using descriptive statistics (number of patients, mean, standard deviation, minimum, maximum, and mean change from Screening). Shift tables (low, normal, high) between Screening and post-Screening timepoints will be presented by laboratory test and cohort. Laboratory tests with categorical results that cannot be analyzed by change from baseline or shift table analysis will not be included in these summaries, but will be listed.

In addition, for all clinical laboratory tests when possible to convert a conversion to NCI-CTCAE grades will be done and summaries based on NCI-CTAE grades will be presented, by actual values, grading, and by shift tables from baseline to worst value during the trial for individual patients.

Data obtained from laboratory tests not required by the protocol will not be summarized, but will be listed.

Descriptive statistics of vital signs and ECG results at each visit will be presented by cohort and will be listed. Shift tables (low, normal, high) between Screening and post-Screening timepoints will be presented by vital sign assessment and cohort.

Concomitant medications will be summarized by cohort and data will be listed.

Physical examination findings will only be listed for each patient.

8.6. Immunogenicity

The immunogenicity testing [redacted] will be performed in PBMC collected at the timepoints indicated in the Schedule of Assessments (Table 1) for each patient.

8.7. [redacted]

[redacted]

8.8. Interim Analysis

An interim analysis will be performed [redacted] to assess safety, immune response, and efficacy parameters.

[redacted]

Patients included in Cohort 2b will, depending on recruitment status and follow-up (targeted follow-up is 6 months), be analyzed in association with the first, and/or the second interim analyses.

There are no plans for early termination of the trial due to superiority or futility of this FIH trial of EO2401 based on the interim results. At the interim analysis, descriptive analyses will be provided with no formal statistical testing.

In addition, an update of OS analysis and listings of efficacy and safety parameters for patients who are still alive in the study after the interim analysis will be performed on a regular basis (every 3 to 6 months as appropriate).

Details on the statistical analyses will be given in a separate Statistical Analysis Plan to cover the interim analysis.

9. REFERENCES

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

10. APPENDICES

Appendix 1 – iRANO Criteria

The goal of this modified response criteria is to meaningfully evaluate radiographic response and progression while simultaneously allowing therapies that may have transient effects on contrast enhancement but therapeutic benefit to be treated equally. This is particularly important in the context of platform trials, where many different therapies may be compared against a common control and there is a significant risk of over or under estimating tumor burden with a single evaluation timepoint. By allowing patients to stay on therapy longer, a more comprehensive and accurate assessment of therapeutic benefit can be performed on retrospective examination. A universal set of principles and guidelines, rather than treatment-specific response criteria, may allow us to fully understand the possible therapeutic benefits and potential limitations of promising new therapies for patients with GB.[79]

The main guidance regarding specific measurements for response and progression in this trial (see Table below) is from Ellingson et al., Modified Criteria for Radiographic Response Assessment in GB Clinical Trials[78] and should be followed. However, note in relation to PD that the confirmatory scan is stated to be done more than or equal to 4 weeks after the initial scan showing "preliminary PD," which also allows the integration of the guidance given in Okada et al., iRANO: A Report of the RANO Working Group[77].

Thus, the following adaptations of the RANO criteria, as reflected in the iRANO criteria[77], will be used to assess response for patients treated on this study:

- Potential PsPD: If radiologic imaging shows initial progressive disease (PD), participants who are not experiencing significant clinical decline may be allowed to continue study treatment for up to 3 months. Patients who have radiographic evidence of further progression after up to 3 months, or who decline significantly at any time, will be classified as progressive with the date of disease progression back-dated to the first date that the participant met criteria for progression and such participants will be discontinued from study therapy. Although the kinetics of PsPD due to immune checkpoint blockade among GB patients is currently unknown, 3 months is a reasonable estimate based on: 1) the peak time for X-ray telescope/daily temozolomide-related PsPD is usually within 3 months of completion for GB patients[90] and; 2) 3 months is also the most common timeframe for PsPD observed among patients with advanced melanoma or other solid tumors treated with PD-1/PD(L)-1 immune checkpoint blockade to date.

See also protocol Sections 7.5.1.1 and 7.5.1.2 for additional guidance.

Response	**Criteria**
Complete Response (CR)	Requires all of the following: • Disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks. The first scan exhibiting disappearance of all enhancing measurable and nonmeasurable disease is considered "preliminary CR." If the second scan exhibits measurable enhancing disease with respect to the "preliminary CR" scan, then the response is not sustained, noted as pseudoresponse (PsR), and is now considered "preliminary PD" (note confirmed PD requires at least 2 sequential increases in tumor volume). If the second scan

	continues to exhibit disappearance of enhancing disease or emergence of nonmeasurable disease (less than 10 mm bidimensional product), it is considered a durable CR. **Note**: Patients with nonmeasurable disease only at baseline cannot have CR; the best response possible is SD.
Partial Response (PR)	Requires all of the following: • ≥ 50% decrease in sum of products of perpendicular diameters or ≥ 65% decrease in total volume of all measurable enhancing lesions compared with baseline, sustained for at least 4 weeks. The first scan exhibiting ≥ 50% decrease in sum of products of perpendicular diameters or ≥ 65% decrease in total volume of all measurable enhancing lesions compared with baseline is considered "preliminary PR." If the second scan exhibits PD with respect to the "preliminary PR" scan, then the response is not sustained, noted as PsR, and is now considered "preliminary PD" (note confirmed PD requires at least 2 sequential increases in tumor volume). If the second scan exhibits SD, PR, or CR, it is considered a durable PR and the patient should continue on therapy until confirmed PD is observed • Steroid dose should be the same or lower compared with baseline scan • Stable or improved clinical assessments. **Note**: Patients with nonmeasurable disease only at baseline cannot have PR; the best response possible is SD.
Progressive Disease (PD)	NOTE: see above regarding adaptation according to the iRANO criteria[77] Defined by any of the following: • At least 2 sequential scans separated by ≥ 4 weeks both exhibiting ≥ 25% increase in sum of products of perpendicular diameters or ≥ 40% increase in total volume of enhancing lesions. The first scan exhibiting ≥ 25% increase in sum of products of perpendicular diameters or ≥ 40% increase in total volume of enhancing lesions should be compared to the smallest tumor measurement obtained either at baseline (if no decrease) or best response (on stable or increasing steroid dose) and is noted as "preliminary PD." If the second scan at least 4 weeks later exhibits a subsequent ≥ 25% increase in sum of products of perpendicular diameters or ≥ 40% increase in total volume of enhancing lesions relative to the "preliminary PD" scan, it is considered "confirmed PD" and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD or PR/CR, this scan showing "preliminary PD" is noted as "pseudoprogression" (PsPD), and the patient should continue on therapy until a second increase in tumor size relative to the PsPD scan is observed. Note that any new measurable (> 10 mm ×10 mm) enhancing lesions should not be immediately considered PD, but instead should be added to the sum of bidimensional products or total volume representing the entire enhancing tumor burden

	• In the case where the baseline or best response demonstrates no measurable enhancing disease (visible or not visible), then any new measurable (> 10 mm ×10 mm) enhancing lesions are considered PD after confirmed by a subsequent scan ≥ 4 weeks exhibiting ≥ 25% increase in sum of products of perpendicular diameters or ≥ 40% increase in total volume of enhancing lesions relative to the scan first illustrating new measurable disease. The first scan exhibiting new measurable disease is noted as "preliminary PD." If the second scan at least 4 weeks later exhibits a subsequent ≥ 25% increase in sum of products of perpendicular diameters or ≥ 40% increase in total volume of enhancing lesions relative to the "preliminary PD" scan, it is considered "confirmed PD" and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD, CR, PR, or becomes nonmeasurable, this scan showing "preliminary PD" is noted as PsPD, and the patient should continue on therapy until a second increase in tumor size relative to the "preliminary PD," or PsPD, scan is observed. Note that any new measurable (> 10 mm ×10 mm) enhancing lesions on the subsequent scan following the preliminary PD scan should not be immediately considered confirmed PD, but instead should be added to the sum of bidimensional products or total volume representing the entire enhancing tumor burden • Clear clinical deterioration not attributable to other causes apart from tumor (e.g., seizures, medication adverse effects, therapy complications, stroke, infection) or attributable to changes in steroid dose • Failure to return for evaluation as a result of death or deteriorating condition.
Stable Disease (SD)	Requires all of the following: • Does not qualify for CR, PR, or PD as defined above. Note this also applies to patients that demonstrate PsR when the confirmation scan does not show PD or PsPD when the confirmation scan does not show PR/CR • In the event that corticosteroid dose was increased (for new symptoms/signs) without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that the steroid increase was required because of disease progression, the last scan considered to show SD will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

Appendix 2 – NANO Scale

Scoring assessment is based on direct observation and testing performed during clinical evaluation and is not based on historical information or reported symptoms. Please check 1 answer per domain. Please check "Not assessed" if testing for that domain is not done. Please check "Not evaluable" if a given domain cannot be scored accurately due to pre-existing conditions, co-morbid events and/or concurrent medications.

Patient Identifier: _____
Date Assessment Performed (day/month/year): _____
Study time point (i.e. baseline, cycle 1, day 1, etc):_____
Assessment performed by (please print name): _____

Domains	**Key Considerations**
Gait 0 ☐ Normal 1 ☐ Abnormal but walks without assistance 2 ☐ Abnormal and requires assistance (companion, cane, walker, etc.) 3 ☐ Unable to walk ☐ Not assessed ☐ Not evaluable	• Walking is ideally assessed by at least 10 steps
Strength 0 ☐ Normal 1 ☐ Movement present but decreased against resistance 2 ☐ Movement present but none against resistance 3 ☐ No movement ☐ Not assessed ☐ Not evaluable	• Test each limb separately • Recommend assess proximal (above knee or elbow) and distal (below knee or elbow) major muscle groups • Score should reflect worst performing area • Patients with baseline level 3 function in one major muscle group/limb can be scored based on assessment of other major muscle groups/limb
Ataxia (upper extremity) 0 ☐ Able to finger to nose touch without difficulty 1 ☐ Able to finger to nose touch but difficult 2 ☐ Unable to finger to nose touch ☐ Not assessed ☐ Not evaluable	• Non-evaluable if strength is compromised • Trunk/lower extremities assessed by gait domain • Particularly important for patients with brainstem and cerebellar tumors • Score based on best response of at least 3 attempts
Sensation 0 ☐ Normal 1 ☐ Decreased but aware of sensory modality 2 ☐ Unaware of sensory modality ☐ Not assessed ☐ Not evaluable	• Recommend evaluating major body areas separately (face, limbs and trunk) • Score should reflect worst performing area • Sensory modality includes but not limited to light touch, pinprick, temperature and proprioception • Patients with baseline level 2 function in one major body area can be scored based on assessment of other major body areas

Scoring assessment is based on direct observation and testing performed during clinical evaluation and is not based on historical information or reported symptoms. Please check 1 answer per domain. Please check "Not assessed" if testing for that domain is not done. Please check "Not evaluable" if a given domain cannot be scored accurately due to pre-existing conditions, co-morbid events and/or concurrent medications.

Visual Fields

- 0 ☐ Normal
- 1 ☐ Inconsistent or equivocal partial hemianopsia (≥quadrantanopsia)
- 2 ☐ Consistent or unequivocal partial hemianopsia (≥quadrantanopsia)
- 3 ☐ Complete hemianopsia
- ☐ Not assessed
- ☐ Not evaluable

- Patients who require corrective lenses should be evaluated while wearing corrective lenses
- Each eye should be evaluated and score should reflect the worst performing eye

Facial Strength

- 0 ☐ Normal
- 1 ☐ Mild/moderate weakness
- 2 ☐ Severe facial weakness
- ☐ Not assessed
- ☐ Not evaluable

- Particularly important for brainstem tumors
- Weakness includes nasolabial fold flattening, asymmetric smile and difficulty elevating eyebrows

Language

- 0 ☐ Normal
- 1 ☐ Abnormal but easily conveys meaning to examiner
- 2 ☐ Abnormal and difficulty conveying meaning to examiner
- 3 ☐ Abnormal. If verbal, unable to convey meaning to examiner. OR non-verbal (mute/global aphasia)
- ☐ Not assessed
- ☐ Not evaluable

- Assess based on spoken speech. Non-verbal cues or writing should not be included.
- **Level 1**: Includes word finding difficulty; few paraphasic errors/neologisms/word substitutions; but able to form sentences (full/broken)
- **Level 2**: Includes inability to form sentences (<4 words per phrase/sentence); limited word output; fluent but "empty" speech.

Level of Consciousness

- 0 ☐ Normal
- 1 ☐ Drowsy (easily arousable)
- 2 ☐ Somnolent (difficult to arouse)
- 3 ☐ Unarousable/coma
- ☐ Not assessed
- ☐ Not evaluable

- None

Behavior

- 0 ☐ Normal
- 1 ☐ Mild/moderate alteration
- 2 ☐ Severe alteration
- ☐ Not assessed
- ☐ Not evaluable

- Particularly important for frontal lobe tumors
- Alteration includes but is not limited to apathy, disinhibition and confusion
- Consider subclinical seizures for significant alteration

Nayak et al, 2017[76]

Appendix 3 – ECOG and Karnofsky Performance Status

ECOG Performance Status

Grade	Description
0	Fully active, able to carry on all predisease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: As published in Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern cooperative oncology group. *Am J Clin Oncol*. 1982;5:649-655.

Karnofsky Performance Status

Grade	Description
100	Normal no complaints; no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or to do active work
60	Requires occasional assistance, but is able to care for most of his personal needs
50	Requires considerable assistance and frequent medical care
40	Disabled; required special care and assistance
30	Severely disabled; hospital admission is indicated although death not imminent
20	Very sick; hospital admission necessary; active supportive treatment necessary
10	Moribund; fatal processes progressing rapidly
0	Dead

Appendix 4 – New York Heart Association Functional Classification

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

[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
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Appendix 6 – Regulatory, Ethical, and Study Oversight Considerations

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
- Applicable laws and regulations.

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
- 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.

Finances and Insurance

Financing and insurance will be addressed in a separate agreement.

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.

Future Use of Patient Samples

Following the completion of all study testing, the remaining tissue and blood/blood components (e.g., additional analysis on PBMC) may be used for Future Biomedical Research. This research will help to understand response against other tumor-associated antigens not included in the current peptide mix and possibly 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.

Patient Data Protection

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

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

Investigator Documentation Responsibilities

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. All data generated from external sources (e.g., central laboratory, ECG central readers) and transmitted to the Sponsor or designee electronically will 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. The Sponsor, or CRO, will review the supporting source documentation against the data entered into the eCRFs to verify

the accuracy of the electronic data. The Investigator will ensure that corrections are made to the eCRFs and that data queries are resolved in a timely fashion by the study staff.

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.

Electronic Data Capture

The CRO will supply the investigational site with access to a web-based EDC computer system. Edit checks and data logic checks are at the point of entry and are validated according to company standard operating procedures. All data entered into the system are transferred to a secure database maintained by the CRO.

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, at the CRO, and at the Sponsor.

Data entries made in the EDC screens should be completed within 5 days of the patient's study visit and must be supported by source documents maintained for all patients enrolled in the study.

The data collection tool for this study will be the validated electronic system. Patient data necessary for analysis and reporting will entered and transmitted via the electronic system. Clinical data management will be performed in accordance with applicable standards and data cleaning procedures. For data coding (e.g., adverse events [AEs], medication), internationally recognized and accepted dictionaries will be used.

After database lock has been declared, all data will be delivered to the Sponsor.

Study Monitoring and Data Quality Assurance

The Sponsor or Sponsor's designee performs quality control and assurance checks on all clinical studies that it conducts. Before enrolling any patients in this study, the Investigator will review the protocol, the EO2401 Investigator Brochure, the eCRF and instructions for their completion, the procedure for obtaining informed consent, and the procedure for reporting AEs and SAEs. The Sponsor's designee will monitor the conduct of the study at the site and will verify eCRF against source documents. Additionally, the Sponsor's designee will use automated validation programs to help identify missing data, selected protocol deviations, out-of-range data, and other data inconsistencies. Requests for data clarification or correction will be electronically provided to the Investigator for resolution.

In accordance with ICH GCP and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor or Sponsor's designee. Inspection of site facilities (e.g., pharmacy drug storage areas, laboratories) and review of study-related records will occur to

evaluate the study conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

Review of Source Documents

The Investigator agrees that the Study Monitor (and other qualified personnel as appropriate) will be allowed to conduct site visits to the investigational facilities for the purpose of reviewing source records pertinent to the study. The Investigator will make the study files available during monitoring visits. These files will also be available for inspection by representatives of the Sponsor, competent authorities and/or the IRB. Patients will not be identified by name on any of the study documents utilized by the Sponsor for their analysis, and confidentiality of information in medical records will be preserved. Every effort will be made to maintain the confidentiality of the patient unless disclosure is required by regulations.

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.

Protocol Deviations

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 noncompliance 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. In case of non protocol deviations, data will not be excluded from the data analysis.

All decisions regarding the type of deviations (important or non-important) will be made prior to commencing the final analysis on the final locked database. A listing of all patients with protocol deviations will be maintained by the Sponsor and a listing of all important protocol deviations will be presented in the final study report.

Investigational sites will report protocol deviations to their IEC/IRB per institutional reporting requirements.

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.

Criteria for Termination of the Study or Study Center/Site

Early termination of the study

If the Sponsor or its designee, the Investigator, or regulatory agency discovers any condition arising during the study that indicate that the study or the study site should be terminated, this action may be taken after appropriate consultation between the Sponsor or its designee and the Investigator. The Sponsor or its designee has the right to terminate the participation of either an individual site or the study at any time, for any reason, which may include the following:

- The incidence and severity of AEs in this or other studies indicates a potential health hazard to patients
- Patient enrollment is unsatisfactory
- Data recording is inaccurate or incomplete
- Investigator(s) do(es) not adhere to the protocol or applicable regulatory guidelines in conducting this study
- Submission of knowingly false information from the study site to the Sponsor or its designee or regulatory authorities.

In the event that the study is terminated early, the Sponsor or its designee will provide specific guidance to investigational sites regarding the end of study procedures.

Clinical Study Report

A clinical study report will be prepared following the completion of the study.

Confidentiality/Disclosure

All information provided regarding the study, as well as all information collected and/or documented during the course of 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.

Records 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 the 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 period of 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's Principal Investigator plans to leave the investigational site so that appropriate arrangements can be made.

Publications

If on completion of the study the data warrant publication, the Investigator may publish the results in recognized (refereed) scientific journals subject to the provisions of the clinical study agreement (CSA). Unless otherwise specified in the CSA, the following process shall occur:

The institution and Investigator shall not publish or present data from an individual study center until the complete multicenter study has been presented in full or for 2 years after the termination of the multicenter study, whichever occurs first. Subsequent publications must refer to the multicenter findings. Thereafter, if the Investigator expects to participate in the publication of data generated from this site, the institution and Investigator shall submit reports, abstracts, manuscripts, and/or other presentation materials to the Sponsor for review before submission for publication or presentation. The Sponsor shall have 60 days to respond with any requested revisions, including (without limitation) the deletion of confidential information. The Investigator shall act in good faith upon requested revisions, except the Investigator shall delete any confidential information from such proposed publications. The Investigator shall delay submission of such publication or presentation materials for up to an additional 90 days in order to have a patent application(s) filed.

Appendix 7 – Treatment-related Reactions

Immediate systemic reactions, infusion-related reactions and hypersensitivity reactions, at administration of nivolumab can be considered to be common (European SmPC and US PI [59, 60] for nivolumab; see also Section 7.6.1.1 and 7.6.1.2 for further guidance), and it can occur also at administration of bevacizumab (European SmPC and US PI[63,69] for bevacizumab; see also Section 7.6.1.3 for further guidance). Systemic reaction at administration of EO2401 cannot be excluded either, and since this is an FIH trial it is reasonable to take precautions to be able to ameliorate symptoms if such would occur.

In the current trial, the nivolumab infusion is to start 3 hours after the EO2401 administration, and when applicable (Cohort 3) the bevacizumab infusion is to start 3 hours after the nivolumab administration. Thus, in the combination therapy parts of the trial, patients will be observed up to 3-4 hours when nivolumab is administered together with EO2401, and 7-8 hours when bevacizumab is also administered. In Cohort 1 where there is administration of EO2401 only, patients will be observed for 3 hours (except the first patient injected who will be observed for 6 hours).

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 and corticosteroids, and epinephrine for intramuscular injection.

Treatment of Administration-related Infusion 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 or 4 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 guidelines (see Section 7.6.3.8.1 for further guidance).

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 premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg orally at least 30 minutes before additional administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, nonsteroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for ≤ 24 hours).

- Stop the infusion, begin an IV infusion of normal saline, and treat the patient with diphenhydramine 50-mg IV (or equivalent) and/or acetaminophen 325- to 1000-mg PO; remain at bedside and monitor patient until resolution of symptoms. Corticosteroid and/or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor patient closely. If symptoms recur, then no further drug will be administered at that visit.

- For future infusions, the following prophylactic premedications are recommended: diphenhydramine 50 mg (or equivalent) and/or acetaminophen/paracetamol 325- to 1000-mg PO should be administered at least 30 minutes before infusions. If necessary, corticosteroids (up to 25-mg IV of SoluCortef 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 subject 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 (depending on cohort of the trial; see Section 3.1).

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).