A Study of Fondazione IRCCS Istituto Nazionale dei Tumori

NIVOLUMAB plus IPILIMUMAB and TEMOZOLOMIDE in combination in microsatellite stable (MSS), *MGMT* silenced metastatic colorectal cancer (mCRC): the MAYA study

Protocol Number: CA209-8U4

Eudract Number: 2018-004299-37

Sponsor: Fondazione IRCCS Istituto Nazionale dei Tumori

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This study will be conducted according to the accepted standards of "Good Clinical Practice"

Reference Centre: s.c. Oncologia Medica 1, Fondazione IRCCS Istituto Nazionale dei Tumori, via G. Venezian n. 1, 20133 Milano

Fondazione IRCCS Istituto Nazionale dei Tumori – Milano
PROTOCOL N° INT ……...

APPROVATO DAL COMITATO ETICO IN DATA

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CLINICAL RESPONSIBLE SIGNATURE

	Signature	**Date**
prof……..		
Responsible Investigator	_____	___/___/___

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A Study of Fondazione IRCCS Istituto Nazionale dei Tumori

[MULTICENTER TRIAL]

CLINICAL SITE SIGNATURE LIST

	Signature	**Date**
Dr…….. Principal Investigator	_____	___/___/___
Dr…….. Sub-Investigator	_____	___/___/___
Dr…….. Sub Investigator	_____	___/___/___
Dr…….. Sub Investigator	_____	___/___/___
Dr…….. Sub Investigator	_____	___/___/___

I have thoroughly read and reviewed the study protocol MAYA Trial.
Having read and understood the requirements and conditions of the study protocol, I agree to perform the clinical study according to the international good clinical practice principles and regulatory authority requirements for source document verification and auditing/inspection of the study. I agree to use the study material, including medication, only as specified in the protocol. I understand that any violation of the protocol may lead to early termination of the study. I agree to the following time schedule. The study will start on January 2019 and is foreseen to be completed by July 2021.

Protocol summary

TITLE: NIVOLUMAB plus IPILIMUMAB and TEMOZOLOMIDE in combination in microsatellite stable (MSS), *MGMT* silenced metastatic colorectal cancer (mCRC): the MAYA study

PI: Dr. Filippo Pietrantonio

PROTOCOL NUMBER: CA209-8U4

VERSION NUMBER: 2.0

Eudract Number: 2018-004299-37

TEST PRODUCT: NIVOLUMAB (NIVO); Ipilimumab (IPI); temozolomide (TMZ)

PHASE: II

INDICATION: Confirmed metastatic or inoperable adenocarcinoma of the colon and/or rectum, with centrally confirmed mismatch repair proficiency (microsatellite stable [MSS]) by multiplex polymerase chain reaction (PCR), *MGMT* promoter methylation by pyrosequencing and MGMT absent expression by immunohistochemistry**.**

SPONSOR: Fondazione IRCCS Istituto Nazionale dei Tumori

Background

In advanced colorectal cancer (CRC), the occurrence of chemorefractory disease poses a major therapeutic challenge due to the presence of an adequate performance status to potentially receive further treatments. Patients who progress after all approved treatments may be generally considered suitable for new investigational drugs or strategies. Thus, in the era of personalized medicine, tumor molecular profiling may lead to the identification of therapeutic targets or predictive biomarkers for pharmacological intervention. The DNA repair gene O^6-methylguanine-DNA methyltransferase (*MGMT*) is responsible of the elimination of alkyl groups from the O^6-position of guanine. If inactive, it may be involved in early steps of colorectal tumor genesis leading to an increase of G- to-A point mutations. Epigenetic silencing of *MGMT* during colorectal tumor genesis is associated with hypermethylation of the CpG island in its promoter. This transcriptional gene silencing is responsible for diminished DNA-repair of O^6-alkylguanine adducts, with the consequence of enhancing chemosensitivity to alkylating agents including dacarbazine and its oral prodrug temozolomide (TMZ). In previous phase II studies, we and others showed that TMZ induced an average objective response rate by RECIST criteria in 10% of heavily pre-treated patients with advanced CRC carrying *MGMT* promoter methylated tumors. Thus, *MGMT* methylation by methylation-specific PCR (MSP) used for patient screening seemed to be a necessary but not sufficient condition to identify response to TMZ. To refine patient selection for TMZ therapy, we showed how digital PCR quantification of *MGMT* methylation may further refine patient selection, with benefit restricted to those with highly hyper-methylated tumors. Moreover, we showed that MGMT negative/low expression by immunohistochemistry (IHC) is found in about one third of methylated samples and is associated with increased response rate. However, even in responding metastatic colorectal cancer (mCRC) patients, acquired resistance to single agent TMZ emerges rapidly and almost invariably within 6 months from treatment initiation.

Immune checkpoint inhibitors have been shown to trigger durable antitumor effects in a subset of patients. A high number of tumor mutations (so called 'tumor mutational burden') have recently been found associated with increased immunogenicity (due to a high number of neoantigens) and improved treatment efficacy across several different solid tumors. Early clinical testing indicated that only 1 of 33 CRC patients had a response to anti PD-1 treatment, in contrast to substantial fractions of patients with melanomas, renal-cell cancers, and lung tumors who showed benefit from PD-1 blockade. Similarly, anti CTLA-4 treatment has until now brought unsatisfactory results in unselected mCRC patients. The probability of response has been ascribed to a high mutational burden (namely an elevated number of somatic mutations), which translates into an increased number of neo-antigens. In mCRCs, only a small fraction of tumors (<5%) display a high mutational load and are usually associated with inactivation of mismatch repair genes such as *MLH1*, *MSH2* and *MSH6*. Molecular alterations in these genes occur as an initial step in colontumor genesis leading to the microsatellite instability (MSI) phenotype. Indeed, mismatch repair– deficient (dMMR) colorectal cancers have 10 to 100 times as many somatic mutations as mismatch repair–proficient colorectal cancers. Moreover, mismatch repair–deficient cancers contain prominent lymphocyte infiltrates, a finding consistent with an immune response. Thus, checkpoint inhibitors may have increased activity in dMMR/microsatellite instability-high (MSI-H) tumors, a hypothesis which was tested in various Phase II trials with positive results. On the contrary,mismatch repair proficient colorectal cancer is unresponsive to immune checkpoint inhibitors.

Hypothesis/rationale

Previous reports indicate that acquired resistance to TMZ may emerge through the induction of a microsatellite-instability-positive phenotype. On the other hand, TMZ by itself has been shown to induce an increase of mutational load in other MGMT deficient solid tumors such as melanoma or glioblastoma. In parallel, other studies have demonstrated that alkylating agents' side effects can influence the immune cell compartment by selectively depleting immuno-suppressive T regulator

lymphocytes (Tregs) and activating immuno-active T cytotoxic lymphocytes (Tc) and natural killers (NK). We recently showed that inactivation of MMR, driven by acquired resistance to the clinical agent temozolomide, increased mutational load, promoted continuous renewal of neoantigens in human colorectal cancers and triggered immune surveillance in mouse models.

On these grounds, we hypothesize that treatment of microsatellite stable *MGMT* hypermethylated CRCs with alkylating agents could reshape the tumor genetic landscape by increasing the tumor mutational burden either directly (by inducing G>A mutations) or/and indirectly (by inactivating DNA repair genes such as *MLH1*, *MSH2* or *MSH6*, which in turn could lead to hypermutated phenotype) therefore enhancing formation of cancer neoantigens and immunogenicity. We also hypothesize that TMZ treatment can modulate the repertoire of immune cells (Tregs, Tc, NK) favoring T cell activation. To achieve potential sensitization to immunotherapy by means of TMZ-induced MSI-like status, treatment with TMZ should be active (i.e. inducing a SD/PR/CR).

Objectives

The **primary objective** is:

- To evaluate the efficacy, measured as 8-month PFS rate, of the combination of TMZ, nivolumab (NIVO) and ipilimumab (IPI) in patients achieving disease control following 2-month lead-in treatment with single agent TMZ.

The **secondary objectives** are:

- To estimate the overall response rates (ORR) of the combination regimen of TMZ, NIVO and IPI, as measured by RECIST 1.1 and immune-related RECIST (ir-RECIST) criteria.
- To estimate duration of response (DoR) of the combination regimen of TMZ, NIVO and IPI.
- To estimate overall survival (OS) of the combination regimen of TMZ, NIVO and IPI.
- To estimate ORR, DoR and PFS according to an Imaging Independent Central Review, using RECIST 1.1 and ir-RECIST criteria.
- To evaluate the safety profile and adverse events encountered by patients treated with the combination of TMZ, NIVO and IPI.
- To assess the quality of life as measured by EORTC QLQ-C30 EORTC QLQ-CR29 and EuroQol EQ-5D.

The **explorative objectives** are:

- To evaluate the relationship between tumor and plasma biomarkers (including but notlimited to PD-L1, PD-1, methyl-BEAMing, mutational load) and efficacy.
- To evaluate the utility of biopsy at the time of enrollment and/or disease progression to characterize primary or acquired resistance to study treatments.

Study Design

Description of Study

This is a Phase II, multicenter, single-arm trial designed to evaluate the efficacy and safety of NIVO, IPI and TMZ combination in 27 patients with microsatellite stable (MSS), MGMT-silenced mCRC with initial clinical benefit following lead-in treatment with single-agent TMZ. Tumor specimens from patients meeting eligibility criteria will be evaluated for sufficient amounts of viable tumor. Only patients whose tumors have sufficient amounts of viable tumor will be enrolled. Specimens will be centrally tested at the Pathology Department of the Fondazione IRCCS Istituto Nazionale dei Tumori for concomitant presence of all the following:

1. Confirmation of MGMT absent expression by IHC
2. Confirmation of MGMT promoter methylation by pyrosequencing

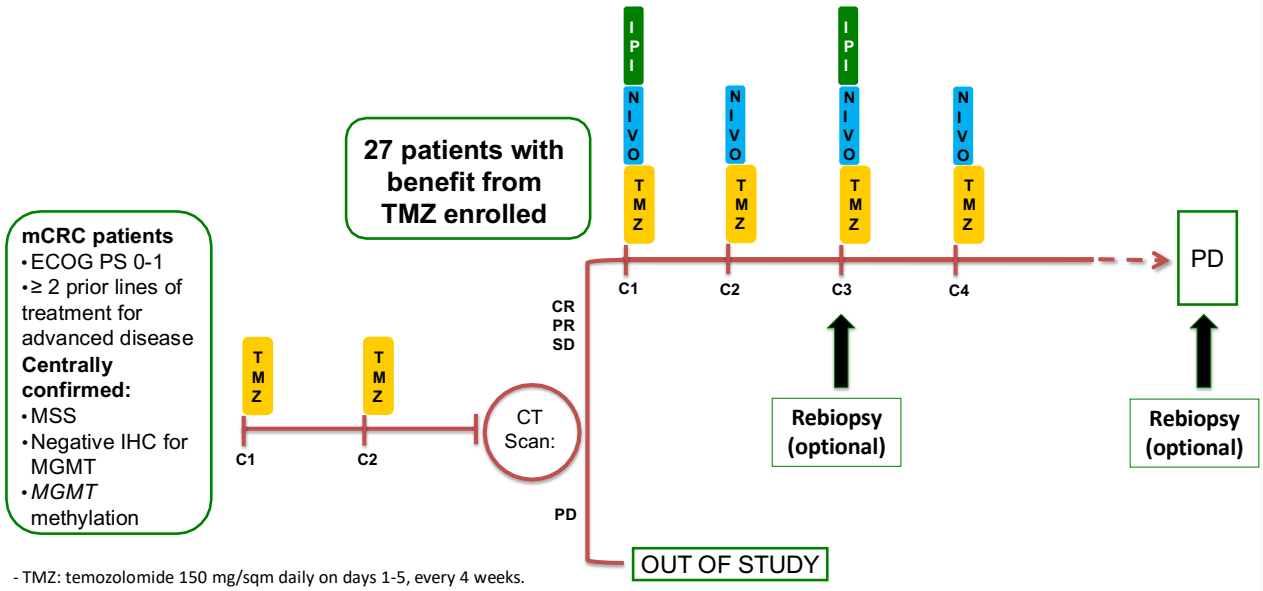
3. Confirmation of microsatellite stable (MSS) status according to multiplex polymerase chain reaction (PCR).

The following algorithm will be followed: first, IHC for MGMT expression will be performed. Only in case of absent expression will pyrosequencing and multiplex PCR be performed.

Note: A separate tissue screening informed consent form shall be used to obtain consent to send the sample to the central laboratory. Subjects may continue on prior therapy while tissue testing takes place.

After central confirmation of eligibility (which will be provided by the Sponsor within 7 +/- 3 days from the arrival of tumor specimens), patients who meet the eligibility criteria will be enrolled. Study design is depicted in Figure 1.

Figure 1. Study Treatment



In the lead-in treatment phase, patients will be treated with:

- TMZ: 150 mg/sqm daily on days 1-5 every 4 weeks, for two cycles.

At the end of the first treatment phase, patients will undergo a CT scan at week 7 +/- 5 days, based on which:

- Patients with progressive disease (PD) according to RECIST v1.1 will be out of study.
- Twenty-seven patients with RECIST v1.1 complete response (CR)/partial response (PR)/stable disease (SD) will be evaluable in the ITT population and will enter the second treatment phase, being treated with the following NIVO+IPI+TMZ combination regimen:
  - NIVO: 480 mg i.v. every 4 weeks.
  - IPI: 1 mg/Kg i.v. every 8 weeks.
  - TMZ: 150 mg/sqm daily on days 1-5, every 4 weeks.

Treatment will continue until confirmed disease progression, unacceptable toxicity, withdrawal of consent or death, whichever occurs first.

- In case of unacceptable toxicity unequivocally due to TMZ, NIVO and IPI can be continued until disease progression, or to unacceptable toxicity, informed consent withdrawal or death.
- In case of unacceptable toxicity unequivocally due to NIVO and/or IPI, single-agent TMZ can be continued until disease progression, or to unacceptable toxicity, informed consent withdrawal or death.

In the second treatment phase, patients will undergo tumor assessment at baseline and every 8 +/- 1 weeks for the first 12 months following Cycle 1, Day 1. After 12 months, patients will undergo tumor assessments every 12 weeks until confirmed disease progression, unacceptable toxicity, withdrawal of consent or death, whichever occurs first.

Patients entering the second treatment phase can continue treatment after the first occurrence of unequivocal radiographic progression per RECIST v1.1, and until the criteria for progression according to ir-RECIST are met. After discussion with the Sponsor, patients will be permitted to continue study treatment after ir-RECIST criteria for progressive disease are met if they meet all of the following criteria:

- Evidence of clinical benefit (defined as the stabilization or improvement of disease-related symptoms) as assessed by the investigator
- Tolerance of study treatment
- Absence of symptoms and signs (including worsening of laboratory values; [e.g., new or worsening hypercalcemia]) indicating unequivocal progression of disease, despite a rising CEA level
- No decline in ECOG performance status that can be attributed to disease progression
- Absence of tumor progression at critical anatomical sites (e.g., leptomeningeal disease) that cannot be managed by protocol−allowed medical interventions
- Patients must provide written consent to acknowledge deferring other treatment options in favor of continuing study treatment at the time of ir-RECIST - defined disease progression. All other elements of the main consent including description of reasonably foreseeable risks or discomforts, or other alternative treatment options will still apply.
- Sponsor approval

Patients who discontinue from study treatment for reasons other than disease progression (e.g., toxicity) will continue scheduled tumor assessments until disease progression, withdrawal of consent or death, whichever comes first. Patients who start a new anti-cancer therapy in the absenceof disease progression should be followed according to the protocol schedule until there is confirmed disease progression, withdrawal of consent or death, whichever occurs first.

CD-ROM copies of the CT scans performed at baseline and during treatment until disease progression according to RECIST v1.1 and ir-RECIST criteria will be collected at the Coordinating Center (s.c. Oncologia Medica 1, Fondazione IRCCS Istituto Nazionale dei Tumori) for central review.

Sites should follow their local privacy practices to de-identify all subject identifying information (name, medical record number, act.) prior to submitting images to the Coordinating Center.

Upon receipt, the Coordinating Center will verify that this information has been completely redacted, and, if necessary, will redact any remaining identifying information.

The sample of archived tumor tissues used for molecular screening, as well as blood and plasma samples, will be collected for exploratory biomarker assessments.

Patients will undergo an optional tumor biopsy sample collection, if clinically feasible as assessed by investigators, prior to C3D1 of the second treatment phase and/or at the evidence of radiographic disease progression causing treatment discontinuation. These data will be analyzed for the

association between changes in tumor tissue and clinical outcome and to understand further the potential mechanisms of resistance to study treatment. A separate informed consent will be signed for optional biopsies requested by the study.

Longitudinal plasma samples (liquid biopsy and PBMCs) will be collected at baseline and every 4 weeks until best response according to ir-RECIST; after that, these samples (liquid biopsy and PBMCs) will be collected every 8 +/-1 weeks for the first 12 months and every 12 weeks thereafter and at disease progression leading to treatment discontinuation.

Safety assessments will include the incidence, nature, and severity of adverse events, changes in vital signs and laboratory abnormalities graded as per National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.0.

Quality of life will be assessed through Patient reported outcomes instruments (PRO) distributed by the study staff and completed in their entirety by the patient. PRO questionnaires should beperformed every 8 ± 1 weeks during treatment until PD, regardless of whether study treatment has been administered or held, and also if the patient is off treatment due to reason other than PD (i.e. refusal or unacceptable toxicity). After 12 months of treatment, PRO questionnaires will be performed every 12 weeks.

Post-progression, all patients will be followed for survival and subsequent anti-cancer therapy approximately every 3 months until death, loss to follow-up or withdrawal of consent, whichever occurs first.

Number of Patients

The study is expected to screen approximately 400 patients to enroll approximately 100 patients who meet the eligibility criteria in the first lead-in treatment phase and 27 patients in the combination treatment phase.

Target Population

Patients must meet the following criteria for study entry:

1. Have provided written informed consent prior to any study specific procedures.
2. Willing and able to comply with the protocol.
3. ≥18 years of age.
4. ECOG status 0 – 1.
5. At least 12 weeks of life expectancy at time of entry into the study.
6. Histologically confirmed metastatic or inoperable adenocarcinoma of the colon and/or rectum, with centrally confirmed mismatch repair proficiency (microsatellite stable [MSS]) by multiplex polymerase chain reaction (PCR), MGMT promoter methylation by pyrosequencing and MGMT absent expression by IHC.
7. Patients with progressive disease or that are not candidate for oxaliplatin irinotecan fluoropyrimidine based chemotherapy and anti EGFR mAbs (in RAS/BRAF wild type tumors) in the metastatic setting.
8. Patients with documented disease relapsed within 6 months from the completion of adjuvant oxaliplatin-based chemotherapy are considered eligible.
9. Measurable, unresectable disease according to RECIST 1.1. Subjects with lesions in a previously irradiated field as the sole site of measurable disease will be permitted to enroll provided the lesion(s) have demonstrated clear progression and can be measured accurately.
10. Is willing and able to provide an adequate archival tumor sample (formalin fixed paraffin-embedded [FFPE]) available for tissue screening for central tissue screening. If the tumor

block is not available, a minimum of twentyfive 3-micron unstained sections on charged slides of tumor will be required.

Patients who meet any of the following criteria will be **excluded** from study entry:

1. Requirement for treatment with any medicinal product that contraindicates the use of any of the study medications, may interfere with the planned treatment, affects patient compliance or puts the patient at high risk for treatment-related complications.
2. Inability to swallow pills.
3. Refractory nausea and vomiting, malabsorption, external biliary shunt or significant bowel resection that would preclude adequate absorption.
4. Inadequate hematological function indicated by all of the following:
   - White Blood Cell (WBC) count < 2 x 10^9/L
   - Absolute neutrophil count (ANC) < 1.5 x 10^9/L
   - Platelet count < 100 x 10^9/L
   - Hemoglobin < 9 g/dL (patients may have transfusions and/or growth factors to attain adequate Hb)
5. Inadequate liver function indicated by all of the following:
   - Total bilirubin ≥ 1.5 x upper limit of normal (ULN)
   - Aspartate transaminase (AST) and alanine aminotransferase (ALT) ≥ 3 x Upper Limit of Normal (ULN) (≥ 5 x ULN in patients with known liver metastases)
   - Alkaline phosphatase (ALP) ≥ 2 x ULN (≥ 5 x ULN in patients with known liver metastases)
6. Inadequate renal function indicated by all of the following:
   - Serum creatinine > 1.5 x ULN or calculated creatinine clearance < 40 ml/min
7. International Normalized Ratio (INR) > 1.5 and activated Partial Thromboplastin Time (aPTT) > 1.5 x ULN within 7 days prior to the start of study treatment for patients not receiving anti-coagulation.
   a. NOTE: The use of full-dose oral or parenteral anticoagulants is permitted as long as the INR or aPTT is within therapeutic limits (according to the medical standard ofthe enrolling institution) and the patient has been on a stable dose of anticoagulants for at least two weeks prior to the start of study treatment
8. Active infection requiring intravenous antibiotics at the start of study treatment.
9. Previous or concurrent malignancy, except for adequately treated basal or squamous cell skin cancer, superficial bladder cancer, or carcinoma *in situ* of the prostate, cervix, or breast, or other cancer for which the patient has been disease-free for three years prior to study entry.
10. Evidence of any other disease, neurologic or metabolic dysfunction, physical examination finding or laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of any of the study medications, puts the patient at higher risk for treatment-related complications or may affect the interpretation of study results.
11. Clinically significant (i.e. active) cardiovascular disease, for example cerebrovascular accidents ≤ 6 months prior to start of study treatment, myocardial infarction ≤ 6 monthsprior to study enrolment, unstable angina, New York Heart Association (NYHA) Functional Classification Grade II or greater congestive heart failure, or serious cardiac arrhythmia uncontrolled by medication or potentially interfering with protocol treatment.

12. History or evidence upon physical or neurological examination of central nervous system (CNS) disease (e.g. seizures) unrelated to cancer unless adequately treated with standard medical therapy.
13. Active brain metastases or leptomeningeal metastases. Subjects with brain metastases are eligible if these have been treated and there is no magnetic resonance imaging (MRI except where contraindicated in which CT scan is acceptable) evidence of progression for at least 8 weeks after treatment is complete and within 28 days prior to first dose of study drug administration. Cases should be discussed with the medical monitor. There must also be no requirement for immunosuppressive doses of systemic corticosteroids (>10mg/day prednisone equivalents) for at least 2 weeks prior to study drug administration.
14. Surgical procedure (including open biopsy, surgical resection, wound revision, or any other major surgery involving entry into a body cavity) or significant traumatic injury within 28 days prior to start of study treatment, or anticipation of need for major surgical procedure during the course of the study.
15. Treatment with any chemotherapy, curative intent radiation therapy, biologics for cancer, or investigational therapy within 28 days of first administration of study treatment (subjects with prior cytotoxic or investigational products < 4 weeks prior to treatment might be eligible after discussion between investigator and sponsor if toxicities from the prior treatment have been resolved to Grade 1 (NCI CTCAE version 4). Prior focal palliative radiotherapy must have been completed at least 2 weeks before study drug administration.
16. All toxicities attributed to prior anti-cancer therapy other than alopecia and fatigue must have resolved to Grade 1 (NCI CTCAE version 4) or baseline before administration of the first treatment phase (lead-in). Subjects with toxicities attributed to prior anti-cancer therapy which are not expected to resolve and result in long lasting sequelae, such as neuropathy after platinum-based therapy, are permitted to enroll.
17. Known hypersensitivity to any of the study medications or known hypersensitivity or allergy to Chinese hamster ovary cell products or any component of the NIVO formulation.
18. History of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins.
19. History of autoimmune disease including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Guillain-Barré syndrome, multiple sclerosis, vasculitis, or glomerulonephritis (see Appendix IV for a more comprehensive list of autoimmune diseases).
    a. Note: Subjects with a history of autoimmune-related hypothyroidism on a stabledose of thyroid replacement hormone may be eligible. Subjects with controlled typeI diabetes mellitus on a stable insulin regimen, vitiligo or psoriasis not requiring systemic treatment may be eligible.
20. Prior allogeneic bone marrow transplantation or prior solid organ transplantation.
21. History of idiopathic pulmonary fibrosis (including pneumonitis), drug-induced pneumonitis, organizing pneumonia (i.e., bronchiolitis obliterans, cryptogenic organizing pneumonia), or evidence of active pneumonitis on Screening chest CT scan.

22. Treatment with systemic immunostimulatory agents (including but not limited to interferons or interleukin-2) within 4 weeks or five half-lives of the drug, whichever is shorter, prior to start of study treatment.
23. Treatment with systemic corticosteroids (>10 mg daily prednisone equivalents) or other systemic immunosuppressive medications (including but not limited to prednisone, dexamethasone, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-tumor necrosis factor [TNF] agents) within 2 weeks prior to start of study treatment, or requirement for systemic immunosuppressive medications during the trial. The use of inhaled corticosteroids and mineralocorticoids (e.g., fludrocortisone) is allowed.
    a. Note: Patients who have received acute, low-dose, systemic immunosuppressant medications (e.g., a one-time dose of dexamethasone for nausea) may be enrolled in the study after discussion with and approval by the Sponsor.
24. Positive test for human immunodeficiency virus (HIV)
25. Active hepatitis B (defined as having a positive hepatitis B surface antigen [HBsAg] test prior to randomization) or hepatitis C.
    a. Note: Patients with past hepatitis B virus (HBV) infection or resolved HBV infection (defined as having a negative HBsAg test and a positive antibody to hepatitis B core antigen antibody test) are eligible. Patients with detectable HBV-DNA are not eligible.
    b. Note: Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction testing is negative for HCV ribonucleic acid (RNA).
26. Active tuberculosis.
27. Administration of a live, attenuated vaccine within 4 weeks prior to start of study treatment or anticipation that such a live attenuated vaccine will be required during the study.
28. Prior treatment with CD137 agonists, anti-CTLA4, anti-PD-1, or anti-PD-L1 therapeutic antibody or drug specifically targeting T-cell co-stimulation or immune checkpoint pathways, including prior therapy with anti-tumor vaccines.
29. Pregnancy or lactation. A serum pregnancy test is required within 7 days prior to start of study treatment, or within 14 days with a confirmatory urine pregnancy test within 7 days prior to start of study treatment
30. For women who are not post-menopausal (< 12 months of non-therapy-induced amenorrhea) or surgically sterile (absence of ovaries and/or uterus): refusal to use a highly effective contraceptive method (i.e. with a failure rate of < 1% per year such as sexual abstinence, hormonal implants, combined oral contraceptives, vasectomized partner), during the study drug administration and for at least 6 months after the last dose of study medication. Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable methods of contraception. A combination of male condom with cap, diaphragm or sponge with spermicide (double barrier methods) is not considered a highly effective birth control method. Acceptable methods of contraception may include total abstinence in cases where the lifestyle of the patient ensures compliance. A vasectomized partner is a highly effective birth control method provided the partner is the sole sexual partner of the trial participant and that the vasectomized partner has received medical assessment of the surgical success.

31. For men: refusal to use a highly effective contraceptive method (i.e. with a failure rate of < 1 % per year such as vasectomy, sexual abstinence or female partner use of hormonal implants or combined oral contraceptives) during the study drug administration and for a period of at least 6 months after the last dose of study medication. Periodic abstinence [e.g., calendar, ovulation, symptothermal, post ovulation methods] and withdrawal are not acceptable methods of contraception. A combination of male condom with either, cap, diaphragm or sponge with spermicide (double barrier methods) is not considered a highly effective birth control methods. Acceptable methods of contraception may include total abstinence in cases where the lifestyle of the patient ensures compliance. A vasectomized trial participant is a highly effective birth control method provided that the trial participant has received medical assessment of the surgical success.

Length of Study

24 months of enrolment and 12 months of follow up

End of Study

The end of study is defined as the date of the last follow-up visit of the last patient enrolled and is expected to occur approximately 12 months after the last patient enrolled in the study. Follow-up for survival will continue until all patients have died or are lost to follow-up or the Sponsor decides to end the trial, whichever occurs first.

Efficacy Outcome Measures

The efficacy outcome measures for this study are as follows:

- Investigator-assessed PFS according to RECIST v1.1
- Investigator-assessed PFS according to ir-RECISTThe

secondary efficacy outcome measures are as follows:

- Investigator - assessed response rate per RECIST v1.1 or death from any cause on study
- Investigator - assessed response rate per ir-RECIST or death from any cause on study
- Investigator - assessed DOR per RECIST v1.1
- Investigator - assessed DOR per ir-RECIST
- OS
- Centrally-assessed objective response, DOR, PFS according to RECIST vers 1.1 and ir-RECIST

Safety Outcome Measures

The safety outcome measures for this study are as follows:

- • Incidence, nature, and severity of adverse events, graded according to the NCI CTCAE v4.0
- • Changes in vital signs, physical findings, and clinical laboratory results

A Data Monitoring Committee (DMC) will be used to evaluate safety during the study. Safety data will be reviewed by the DMC on a periodic basis, approximately every 3 months from the date of first-patient-in. In addition, the DMC will review safety data 28 days after the inclusion of the 6th patient. Safety data, including demographics, adverse events, serious adverse events, and relevant laboratory data, will be reviewed.

The DMC will provide a recommendation as to whether the study may continue, whether amendment(s) to the protocol should be implemented, or whether the study should be stopped. The final decision will rest with the Sponsor.

Patient-Reported Outcome Measures

The PRO outcome measures for this study are as follows:

- EORTC QLQ-C30
- EORTC QLQ-CR29
- EuroQol EQ-5D

Exploratory Outcome Measures

The exploratory outcome measures for this study are as follows:

Longitudinal plasma samples (liquid biopsy and PBMCs) will be collected at baseline and every 4 weeks until best response according to ir-RECIST; after that, these samples (liquid biopsy and PBMCs) will be collected every 8 +/-1 weeks for the first 12 months and every 12 weeks thereafter and at disease progression leading to treatment discontinuation. Patients will undergo an optional tumor biopsy sample collection, if clinically feasible as assessed by investigators, prior to C3D1 of the second treatment phase and/or at the evidence of radiographic disease progression causing treatment discontinuation. A separate informed consent will be signed for optional biopsies requested by the study.

Quantification of the percentage of MGMT methylation (by digital PCR based methods, i.e. methyl-BEAMing) and MGMT IHC will be performed in archival tumor tissue and tumor re-biopsies.

Digital PCR for MGMT methylation status will be performed in cell-free circulating DNA (cfDNA) (cfDNA).

Mutational load will be assessed in archival tumor tissues, tumor biopsies and in cell-free circulating DNA (cfDNA) by means of whole exome sequencing.

Immune-related tissue and circulating biomarkers will also be studied.

Investigational Medicinal Products

The investigational medicinal products (IMP) for this study are NIVO, IPI and TMZ. TMZ was used in previous phase II studies for patients with heavily pre-treated advanced CRC carrying *MGMT* promoter methylation and it induced an average objective response rate by RECIST criteria in 10% of cases. Evidence from recent studies demonstrated that NIVO provides a high percentage of durable response in previously treated patients with mismatch repair–deficient mCRC leading to the approval by the FDA for the treatment of patients harboring those characteristics. In preclinical and clinical settings, the combination of NIVO plus IPI has provided enhanced activity over NIVO monotherapy. An accelerated approval was granted by the FDA for this combination on July 10th, 2018, based on findings from the phase II CheckMate-142 trial.

Determination of Sample Size

According to our previously published results, in MGMT methylated mCRC with initial clinical benefit from TMZ (CR+PR+SD at 8 weeks) the individual PFS is almost always shorter than 6 months, and therefore the median PFS falls between 16- and 24-week reassessments. Therefore, after the first 8-week CT scan reassessment, by adding NIVO and IPI to TMZ, we plan to increase the 8-month PFS rate from 0% to 20%.

According to the Fleming single-stage design and selecting the design parameters p0 (8-months PFS rate in the null hypothesis) = 0.05*, and p1 (8-months PFS rate in the alternative hypothesis) =

0.20, respectively, a total of 27 patients will be required. Null hypothesis will be rejected if at least 4 patients will be progression-free at the 8-months reassessment.

*A null hypothesis of 0.05 is based on the consideration that single agent TMZ does not lead to individual PFS times beyond 6 months in the refractory setting.

**The alternative hypothesis of 0.20 is considered as a potential target of interest for further studies in this setting with the experimental treatment.

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1 LIST OF ABBREVIATIONS

ADR	Adverse Drug Reaction
AE	Adverse Event
AESI	Adverse Event of Special Interest
AIDS	Acquired Immunodeficiency Syndrome
ALT (SGPT)	Alanine-Aminotransferase (Serum Glutamic Pyruvic Transaminase)
ALP	Alkaline Phosphatase
ANC	Absolute Neutrophil Count
aPTT	Activated Partial Thromboplastin Time
AST (SGOT)	Aspartate-Aminotransferase (Serum Glutamic Oxalacetic Transaminase)
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CEA	Carcinoembryonic Antigen
cfDNA	cell-free circulating DNA
CNS	Central Nervous System
CR	Complete Remission
CRC	Colorectal cancer
CT	Computed Tomography Scan
CTA	Clinical Trial Agreement
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Anti−cytotoxic T lymphocyte−associated antigen 4
DILI	Drug-Induced Liver Injury
DMC	Data Monitoring Committee
d-MMR	Deficient mismatch repair (dMMR)
DNA	Deoxyribonucleic acid
DOR	Duration of Response
eCRF	Electronic Case Report Form
ECOG PS	Eastern Cooperative Oncology Group-Performance Status
ECG	Electrocardiography
EDC	Electronic Data Capture
EORTC QLQ-C30	European Organization for Cancer Research Treatment Quality of life Instrument
EORTC QLQ-CR29	European Organization for Cancer Research Treatment Quality of life Instrument
EuroQol EQ-5D	EuroQoL 5 Dimensions
EC	Ethics Committee
EGFR	Epidermal Growth Factor Receptor
FOLFOX	FOLinic acid, Fluorouracil and OXaliplatin
FOLFIRI	FOLinic acid, Fluorouracil, IRInotecan
FFPE	Formalin Fixed Paraffin-embedded
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IB	Investigator's Brochure
ICH	International Conference on Harmonisation on Good Clinical Practice
IEC	Independent Ethics Committee
IHC	Immunohistochemistry
INR	International Normalized Ratio
IPI	Ipilimumab
IRB	Institutional Review Board
LDH	Lactate Dehydrogenase
LFT	Liver Function Test(s)
mAB	Monoclonal Antibody
mCRC	Metastatic colorectal cancer
MGMT	O6-methylguanine-DNA methyltransferase
mRECIST	modified Response Evaluation Criteria in Solid Tumors
MRI	Magnetic Resonance Imaging
MSI	Microsatellite Instability

MSS	Microsatellite Stable
MSP	Methylation Specific PCR
MTIC	Monomethyl Triazenoimidazole Carboxamide
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Event
NGS	Next-Generation Sequencing
NIVO	Nivolumab
NK	Natural Killers
NYHA	New York Heart Association
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PD-L1	Programmed Death-Ligand 1
pMMR	Proficient Mismatch Repair
PFS	Progression Free Survival
PR	Partial Response
PRO	Patient Reported Outcome
QTc	Corrected QT
QTcF	Fridericia-corrected QT
RECIST	Response Evaluation Criteria for Solid Tumor, v1.1
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SD	Stable Disease
SOC	Standard of Care
SUSAR	Suspected Unexpected Serious Adverse Reaction
T3	Triiodothyronine
T4	Thyroxine
TCR	T-cell Receptor
TNF	Tumour Necrosis Factor
TRG	T-Cell Receptor Gamma
TSH	Thyroid-stimulating hormone
Tc	Immuno-active T cytotoxic lymphocytes
TMZ	Temozolomide
Tregs	Immuno-suppressive T regulator lymphocytes
ULN	Upper Limit of Normal
WBC	White Blood cell

INTRODUCTION AND STUDY RATIONALE

1.1 Study Rationale

1.1.1 Disease Background

The management of metastatic CRC (mCRC) remains a significant clinical challenge to oncologists worldwide. Even though appropriate screening programs (implementation of fecal occult blood test and colonoscopy) and preventive strategies are in place in many countries, a significant number of patients are still diagnosed at late stages of the disease, resulting in high disease burden and mortality (1) (2).

Although care for patients with CRC has improved greatly in the last 20 years, considerable variation still exists in cancer management and outcome among European countries, and large variation is also apparent between national guidelines and patterns of cancer care in Europe. While surgery remains the mainstay of curative treatment (5-year survival rates of 20–45% reported after the surgical resection of R0 resectable colorectal liver metastases), the management of mCRC must be a multi-modal approach. Long-term survival, maintained quality of life, and even cure can be achieved in selected patients by a combination of optimal choice chemotherapy and surgery (1). The choice of first-line treatment for mCRC is influenced by the clinical presentation and patterns of tumor biology (e.g. metastases limited to liver and/or lung, or peritoneum; dynamics of progression; present or imminent symptoms; prognostic molecular or biochemical markers, such as BRAF V600E mutation), as well as patient-related factors (e.g. co-morbidity and related potential to undergo secondary resection), and drug-related factors (availability of targeted drugs; predictive markers, e.g. RAS mutation) (1).

Pharmacological treatment options for mCRC have increased substantially in the past decade (3). While combinations of fluoropyrimidines with oxaliplatin or irinotecan (such as in 5-FU, leucovorin and oxaliplatin [FOLFOX] / capecitabine and oxaliplatin [XELOX] or 5-FU, leucovorin and irinotecan [FOLFIRI] 5-FU, leucovorin, oxaliplatin and irinotecan [FOLFOXIRI]) constitute the main treatment of choice in this patient population (2), the addition of novel biological therapies targeting cancer-specific molecules to these cytotoxic agents have led to significantly improved outcomes and is the current standard treatment formCRC (4); (2)). A broad variety of molecular targeting agents are currently available, such as anti- angiogenic agents (bevacizumab) and epidermal growth factor receptor (EGFR) antibodies (cetuximab, panitumumab) for first-line treatment of mCRC. A recent systematic review of randomized controlled trials concluded that the oral fluoropyrimidine formulation, capecitabine had similar outcomes and better safety than 5-fluorouracil (5-FU) with oxaliplatin but not irinotecan; first-line oxaliplatin and irinotecan appear equivalent; and antiangiogenics (bevacizumab, ziv-aflibercept, ramucirumab) and EGFR-targeted monoclonal antibodies (MAb) (cetuximab and panitumumab) further improved the efficacy of fluoropyrimidine-based regimens in the treatment of mCRC (5). However, while metastatic CRC patients have many treatment options, the optimal use and sequence of targeted agents remain to be determined (2). In patients with refractory mCRC, regorafenib and TAS102 provided an incremental and significant, although very modest, benefit of overall survival (6) (7).

1.1.2 Immunotherapy for metastatic colorectal cancer

The significance of the immune system in the biology of CRC is emphasized by retrospective assessments of immune infiltrates in resected CRC tumors. The presence of tumor infiltrating lymphocytes—especially CD8þlymphocytes—in the tumor microenvironment, as well as regional lymph nodes, has been linked to better prognosis (8). Also, increased infiltration of specific regions of the CRC tumors by cytotoxic memory T lymphocytes (CD8þCD45ROþT-cells) is highly correlated with reduced risk of recurrence of CRC and better survival (9) (10). It is also known that the presence of these effector memory T-cells is more importantthan naïve T-cells in reducing the risk of relapse and improving survival. The prognostic significance of T- cells—unlike other inflammatory cells—argues that cancer immunotherapies modulating T-cell responses

could lead to improved survival. Recent developments in immune-biotechnology and the discovery of immunotherapy agents—specifically checkpoint inhibitors— have been promising for treatment of melanoma (11) (12) and non–small cell lung cancer (13), leading to regulatory approval of these novel drugs.

The anti–PD-1 antibody nivolumab (NIVO) did not demonstrate clinically significant activity in a Phase I study of multiple tumor types, which included 17 patients with heavily pre-treated, metastatic CRC. However, six of the seven tumors found in this cohort, which were tested for PD-L1 expression, were negative, potentially explaining the observed lack of response (14). Notably, one patient from this metastatic-CRC cohort, who had a PD-L1 positive tumor and was treated with 5 doses of nivolumab, showed complete response after 6 months and no signs of disease after 3 years. This patient's tumor was also mismatch repair- deficient (15).

Pembrolizumab is a humanized IgG4 monoclonal antibody that blocks the interaction of PD-1 with its ligands PD-L1 and PD-L2. A Phase II trial of pembrolizumab evaluated 32 patients with advanced CRC [11 with deficient mismatch repair (dMMR) and 21 with proficient mismatch repair (pMMR)] (16). All patients enrolled in the study had received two or more (median of four) previous chemotherapy regimens, except one patient who had received one chemotherapy regimen and one non-PD- 1 based immunotherapy before. The immune-related objective response rate and patients with stable disease were significantly higher in the dMMR CRC patients (40% and 78%, respectively) than in the pMMR CRC patients (0% and 11%, respectively). Similar findings were observed in the cohort with dMMR cancers other than CRC. Based on this, a larger Phase II study (Keynote-164) is currently enrolling patients to assess the benefit of pembrolizumab for unresectable or metastatic dMMR CRC refractory to two or more previous lines of therapy (NCT02460198).

The anti–PD-L1 antibody BMS936559 showed no activity in a Phase I trial that included 18 patients with CRC (17). However, preliminary data with another anti–PD-L1 antibody MPDL3280A showed activity in CRC with one of four patients achieving a durable partial response (18). Another Phase Ib trial, assessing MPDL3280A in combination with bevacizumab in refractory CRC patients and in combination with FOLFOX plus bevacizumab in first-line CRC, showed good tolerability and clinical activity [unconfirmed ORR 8% (1/13) and 44% (8/18), respectively] (19).

A dMMR system is present in 10–20% of patients with sporadic CRC and is associated with a favorable prognosis in early-stage disease. In contrast, dMMR occurs in only 3–6% of patients with advanced CRC (20) (21). As the immune system can recognize somatic mutations found in tumors (22), and as colorectal tumors with dMMR have several times as many somatic mutations as proficient MMR tumors (23) (24), it has been hypothesized that the immune system may play a role in dMMR tumors possessing a reduced metastatic potential. The probability of response has been ascribed to a high mutational burden (namely an elevated number of somatic mutations due to inactivation of mismatch repair genes such as MLH1, MSH2 and MSH6.), which translates into an increased number of neo-antigens.

Additionally, dMMR cancers have prominent lymphocyte infiltrates (25), which supports the above hypothesis. Furthermore, recent findings have suggested that the infiltrate in dMMR CRC is more likely to express PD-L1, which may predict response to PD-1 blockade (26). Thus, checkpoint inhibitors may have increased activity in dMMR/microsatellite instability-high (MSI-H) tumors, a hypothesis which was tested in various Phase II trials with positive results (27) (28) (29), (30). Although immunotherapy in dMMR tumors holds great promise, the complete absence of response in patients with pMMR CRC—who represent the vast majority—highlights the ongoing need to understand why patients with conventional CRC lack robust responses to immunotherapy.

1.1.3 Temozolomide (TMZ) for metastatic colorectal cancer with MGMT methylation

In advanced CRC, the occurrence of chemo-refractory disease poses a major therapeutic challenge due to the presence of an adequate performance status to potentially receive further treatments. Patients who progress after all approved treatments may be generally considered suitable for new investigational drugs or strategies. Thus, in the era of personalized medicine, tumor molecular profiling may lead to the identification of therapeutic targets or predictive biomarkers for pharmacological intervention. MGMT is a repair protein that removes alkylating groups from the O^6-guanine in DNA. It protects normal and tumor cells from this type of DNA damage by moving the alkylating group to a cysteine residual within its own protein (31). Approximately 40% of metastatic colorectal cancer (CRC) shows silencing of the *MGMT* gene, leading this to absence of the corresponding protein. Due to this deficiency, the tumor cell is not able to effectively repairO^6-methylguanine adducts, causing a higher frequency of G:C > A:T transitions and potentially enhancing the susceptibility to cytotoxic effect of alkylating agents such as TMZ (TMZ) or dacarbazine (31) (32). MGMT deficiency can be assessed in tumor samples either as promoter hypermethylation by Methyl- Specific PCR (MSP) and digital PCR quantification by next generation sequencing methods such as MethylBEAMing (33) or lack of protein expression by immunohistochemistry (IHC) (34).

While the clinical validation of MGMT as a predictive biomarker has been achieved for melanoma and glioblastoma, where alkylating agents have been the backbone of systemic treatment for years, the same is not the case for metastatic CRC where these drugs are of very limited activity (35) (36) (37). Indeed, only few data are available regarding treatment of CRC with these agents based on MGMT methylation. In particular, some phase II clinical trials have assessed the clinical efficacy of alkylating agents in metastatic CRC based on MGMT deficiency as a biomarker (35) (36) (37). In all these studies, selection was made by assessing promoter hypermethylation by Methyl-Specific PCR (MSP) on formalin-fixed paraffin embedded archival tumor tissue. Response rate was ranging from 4 to 16% in heavily pretreated populations. Even though MSP is a well-standardized assay in melanoma and glioblastoma, in CRC the selection according to this methodology appears to be a useful but not optimal condition for achieving clinical benefit. In fact, we showed that digital PCR quantification of *MGMT* methylation could further refine patient selection, with benefit restricted to those with highly hyper-methylated tumors (33). Moreover, we showed that MGMT low expression at IHC was found in about one third of MSP-methylated samples and was associated with increased response rate (38). Our unpublished observations on a pooled cohort of 105 mCRC patients treated with alkylating agents indicate that assessment of MGMT promoter methylation coupled with reduced protein expression optimizes prediction of response and is strongly associated with improved PFS.

1.1.4 Background on Nivolumab and Ipilimumab in mCRC

The combination of nivolumab and ipilimumab was chosen as an experimental arm because of preclinical and preliminary clinical evidence suggesting synergy between nivolumab and ipilimumab. While PD-1 and CTLA-4 are both co-inhibitory molecules, evidence suggests that they use distinct mechanisms to limit T cell activation. Preliminary indirect data from peripheral T cell assessments suggest that a given T-cell checkpoint inhibitor may modulate host immune cell phenotype rendering them more susceptible to alternatecheckpoint inhibitors and thereby enhancing anti-tumor activity. Specifically, nivolumab increased peripheral CTLA-4+ and regulatory T cells in subjects without clinical response in CA209006. In a preclinical melanoma model, anti-CTLA-4 therapy increased PD-1+, PD-L1+ and CTLA-4+ tumor infiltrating T cells. CTLA-4 knockout mice suffer from a fatal lymphocyte proliferative disorder. CTLA-4 is expressed on Treg cells and transiently on activated T cells. CTLA-4 interaction with its ligands B7-1 (CD80) and B7-2 (CD86), results in cell cycle arrest, with G1 inhibition particularly evident in CD4 cells on secondary antigen exposure. In vivo blockade of CTLA-4, utilizing anti-CTLA-4 mAb, induces regression ofestablished tumors and enhanced antitumor immune responses in several immunogenic murine tumor models. Moreover, when anti-CTLA-4 mAb is used in conjunction with granulocyte macrophage colony

stimulating factor (GM-CSF)-secreting tumor vaccines, poorly immunogenic cancers in mice are rejected. Tumor infiltration with activated lymphocytes, associated inflammation, and increased resistance to Treg activity as well as microenvironmental changes in tumor vasculature, are the hallmark of the anti-tumor effect of ipilimumab. These findings suggest that CTLA-4 blockade, alone or in combination with another immunological agent, can induce a potent antitumor response. In addition, in the Phase 2 ipilimumab monotherapy study CA184004, increases in tumor infiltrating lymphocytes (TILs) and interferon-inducible genes were observed following treatment with ipilimumab, and PD-L1 positive tumor cells co-localize with both TILs and interferon expression in metastatic melanoma. The preliminary clinical evidence has demonstrated a higher frequency of patients with substantial tumor burden reduction for the combination of nivolumab and ipilimumab. Improved overall survival associated with substantial tumor burden reduction has been noted with immunotherapies. For instance, improved overall survival has been noted in metastatic melanoma subjects obtaining a complete response to IL-2. If this observation is also applicable to treatment with nivolumab combined with ipilimumab then there could also be the potential for large improvements in overall survival compared to other targeted therapies or to chemotherapy.

In a multicenter phase 2 trial in patients with advanced colorectal cancer locally assessed as dMMR/MSI-H, NIVO provided durable responses (ORR, 32% per central assessment) and disease control (DCR, 64%) in pre-treated pts with dMMR/MSI-H mCRC (NCT02060188, Checkmate-142) (28). NIVO was approved in the US for patients with dMMR/MSI-H mCRC who progress after standard chemotherapy with a fluoropyrimidine (F), oxaliplatin (Ox), and irinotecan (Iri). Long-term survival and outcomes by prior chemotherapy with Nivolumab in CheckMate-142 were presented at the 2018 ASCO GastroIntestinal Cancers Symposium: in the 74 patients treated with NIVO 3 mg/kg Q2W, overall response rate (ORR) was 34%; complete responses (CRs) increased from 3% in prior database lock (DBL) to 9%. Furthermore, the interim analysis of the NIVO + ipilimumab (IPI) combination cohort of CheckMate-142 reported a preliminary ORR of 55% and manageable safety profile in a subset of patients (n = 84) with dMMR/MSI-H mCRC and ≥ 6 mo of follow-up (39). Efficacy and safety data from the complete population (N = 119) of theNIVO + IPI cohort of CheckMate-142 were presented at the 2018 ASCO GastroIntestinal Cancers Symposium. Patients with dMMR/MSI-H mCRC received NIVO 3 mg/kg + IPI 1 mg/kg Q3W for 4 doses followed by NIVO 3 mg/kg Q2W. Of 119 treated patients, 76% had ≥ 2 prior lines of therapy. Median follow-up was 13.4 mo. The ORR was 55% and DCR was 80%. Notably, ORR in pts with a BRAF mutation was 55%. Among all responders, median DOR was not reached (NR), with 94% of responses ongoing at datacutoff. Tumor burden was reduced from baseline in 77% of pts. The 9-mo PFS and OS rates were 76% and 87%, respectively. Gr 3–4 TRAEs occurred in 32% of pts; 13% (any gr) and 10% (gr 3–4) of pts had TRAEs that led to discontinuation.

Based on these results, the combination of NIVO + IPI has been granted an accelerated approval by the FDA and may represent a new standard of care in patients with dMMR/MSI-H mCRC. The accelerated approval of this combination is contingent upon results from a confirmatory trial.

However, there is a great unmet need for tools to improve patient selection and to increase responses to checkpoint inhibitors for patients affected by pMMR CRC, who represent the majority of CRC patients.

1.1.5 Rationale for TMZ, Nivolumab and Ipilimumab in combination

Previous reports indicate that acquired resistance to TMZ may emerge through the induction of a microsatellite-instability-positive phenotype (40) (41). On the other hand, TMZ by itself has been shown to induce an increase of mutational load in other MGMT deficient solid tumors such as melanoma or glioblastoma (42). In parallel, other studies have demonstrated that alkylating agents' side effects can influence the immune cell compartment by selectively depleting immuno-suppressive T regulator lymphocytes (Tregs) (43), and activating immuno-active T cytotoxic lymphocytes (Tc) and natural killers (NK) (44). In addition, TMZ has been shown to induce immunogenic cell death (45). On these grounds, we

hypothesize that treatment of microsatellite stable *MGMT* silenced CRCs with alkylating agents could reshape the tumor genetic landscape by increasing the tumor mutational burden either directly (by inducing G>A mutations) or/and indirectly (by inactivating DNA repair genes such as MLH1, MSH2 or MSH6, whichin turn could lead to hypermutated phenotype) therefore enhancing formation of cancer neoantigens and immunogenicity. We also hypothesize that TMZ treatment can induce immunogenic cell death and modulate the repertoire of immune cells (Tregs, Tc, NK) favoring T cell activation. Finally, our recently published preclinical and translational data suggest that prolonged TMZ treatment until resistance may induce exponential increase of mutational load and MSI phenotype in *MGMT* silenced CRC. In both cell lines treated with TMZ and patients with acquired resistance to TMZ-based therapies, we showed the emergenceof high mutational load. In the tumor specimens of mCRC patients with MMR proficient tumors collected at treatment progression we observed that resistance to TMZ correlated with inactivation of MMR and increased mutational loads (Germano et al., 2017) iv). TMZ can cause inactivation of DNA repair, increased mutational burden and effective immune surveillance in preclinical models, *de facto* turning a p-MMR tumorinto a 'd-MMR- like' (Germano et al., 2017).

Thus, there is a strong rationale for combining TMZ plus NIVO and IPI in MSS, *MGMT* silenced advanced CRC. In fact, TMZ may be used to prime the sensitivity to immunotherapy of a subset of colorectal cancers which is expected to be refractory to immune checkpoint blockade.

1.2 Research Hypothesis

Treatment with TMZ, NIVO and IPI will have clinical activity in subjects with metastatic MSS, MGMT silenced CRC.

1.3 Objectives

1.3.1 Primary Objective

- To evaluate the efficacy, measured as 8-month PFS rate, of the combination of TMZ, NIVO and IPI in patients achieving disease control following 2-month lead-in treatment with single agent TMZ.

1.3.2 Secondary Objectives

- To estimate the overall response rates (ORR) of the combination regimen of TMZ, NIVO and IPI, as measured by response rate according to RECIST 1.1 and ir-RECIST criteria.
- To estimate duration of response (DoR) of the combination regimen of TMZ, NIVO and IPI.
- To estimate overall survival (OS) of the combination regimen of TMZ, NIVO and IPI.
- To estimate ORR, DoR and PFS according to an Imaging Independent Central Review, using RECIST 1.1 and ir-RECIST criteria.
- To evaluate the safety profile and adverse events encountered by patients treated with the combination of TMZ, NIVO and IPI.
- To assess the quality of life as measured by EORTC QLQ-C30 EORTC QLQ-CR29 and EuroQol EQ-5D.

1.3.3 Exploratory Objectives

- To evaluate the relationship between tumor and plasma biomarkers (including but not limited to PD-L1, PD-1, methyl-BEAMing, mutational load) and efficacy.
- To evaluate the utility of biopsy at the time of enrollment and/or disease progression to characterize primary or acquired resistance to study treatments.

Patients will undergo an optional tumor biopsy sample collection, if clinically feasible as assessed by investigators, prior to C3D1 of the second treatment phase and/or at the evidence of radiographic disease progression causing treatment discontinuation. These data will be analyzed for the association between

changes in tumor tissue and clinical outcome and to understand further the potential mechanisms of resistance to study treatment. A separate informed consent will be signed for optional biopsies requested by the study.

Longitudinal plasma samples (liquid biopsy and PBMCs) will be collected at baseline and every 4-week until best response according to ir-RECIST; after that, these samples (liquid biopsy and PBMCs) will be collected every 8 +/-1 weeks for the first 12 months and every 12 weeks thereafter and at disease progressionleading to treatment discontinuation.

Quantification of the percentage of MGMT methylation (by digital PCR based methods) and MGMT IHC will be performed in archival tumor tissue and tumor re-biopsies. Moreover, digital PCR for MGMTmethylation status will be performed in cell-free circulating DNA (cfDNA). Mutational load will be assessedin archival tumor tissues, tumor biopsies and cfDNA before and during treatment by next-generation sequencing of exome or large gene panels (including DNA mismatch repair genes and microsatellite loci). Custom algorithms will be applied to count the number of G>A or C>T transitions, which are known genomic marks of TMZ exposure. We will then assess and rank the immunogenic value of all nucleotide variants induced by TMZ treatment using available bioinformatics tools (e.g. NetChop Cterm and NetMHC algorithms) that will inform about whether and to what extent chemotherapy-induced molecular alterations could result in the generation of neo-epitopes. Immune-related tissue and circulating biomarkers will also be studied. Analysis of PD-L1 will be performed on archival or fresh pre-treatment biopsies by IHC using the Dako anti PD-L1 antibody clone 28-8. Positivity is defined as membranous PD-L1 expression in more than 5% neoplastic cells. Scoring of PD-L1 staining in tumor-infiltrating lymphocytes (TILs) will also be performed. PD1 and other lymphocyte activation markers will be measured in peripheral blood lymphocyte (PBL) post-treatment and correlated with mutational load. For these analyses, PBL will be isolated at baseline and every four weeks in concomitance with cfDNA extraction and stored frozen until analysis. LymphoTrack® Dx next-generation sequencing (NGS) assays will be used to detect T-cell receptor gamma (TRG) rearrangements in PBL taken post-treatment.

1.4 Product Development Background

The following section references the nivolumab Investigator Brochure (IB).

1.4.1 Nivolumab and Ipilimumab Mechanisms of Action

Cancer immunotherapy rests on the premise that tumors can be recognized as foreign rather than as self and can be effectively attacked by an activated immune system. An effective immune response in this setting is thought to rely on immune surveillance of tumor antigens expressed on cancer cells that ultimately results in an adaptive immune response and cancer cell death. The immune surveillance functions by limiting the emergence of tumors as they arise and/or causing tumor shrinkage. Tumor progression may depend upon acquisition of traits that allow cancer cells to evade immune surveillance and an effective immune response. This evasion may occur by exploiting any of the checkpoints that control the regulatory immune response, including display of antigens and control of co-stimulatory pathways that affect the proliferation of cells involved in immunity. Current immunotherapy efforts attempt to break the apparent tolerance of the immune system to tumor cells and antigens by either introducing cancer antigens by therapeutic vaccination or by modulating regulatory checkpoints of the immune system, either directly by stimulation of immune cells by antibodies directed to receptors on T and B cells or indirectly by cytokine manipulation. T-cell stimulation is a complex process involving the integration of numerous positive, as well as negative, co-stimulatory signals in addition to antigen recognition by the T-cell receptor (TCR). Collectively, these signals govern thebalance between T-cell activation and tolerance to antigens.

Programmed death receptor-1 (PD-1, CD279), a 55 kD type I transmembrane protein, is a member of the CD28 family of T-cell co-stimulatory receptors that also includes CD28, CTLA-4, ICOS, and BTLA.

PD-1 signaling has been shown to inhibit CD-28-mediated upregulation of IL-2, IL-10, IL-13, interferon γ (IFN γ) and Bcl-xL. PD-1 expression has also been noted to inhibit T cell activation, and expansion of previously activated cells. Evidence for a negative regulatory role of PD-1 comes from studies of PD-1 deficient mice, which develop a variety of autoimmune phenotypes. (46)These results suggest that PD-1 blockade has the potential to activate anti-self T-cell responses, but these responses are variable and dependent upon various host genetic factors. Thus, PD-1 deficiency or inhibition is not accompanied by a universal loss of tolerance to self-antigens. Taken together, these results suggest that PD-1 blockade has the potential to activate anti-self T-cell responses, but these responses are variable and dependent upon various host genetic factors. Thus, PD-1 deficiency or inhibition is not accompanied by a universal loss of tolerance to self-antigens.

Preclinical animal models of tumors have shown that blockade by PD-1 by monoclonal antibodies (mAbs) can enhance the anti-tumor immune response and result in tumor rejection. Antitumor activity by PD-1 blockade functions in PD-L1-positive tumors as well as in tumors that are negative for the expression of PD- L1. This suggests that host mechanisms (i.e., expression of PD-L1 in antigen-presenting cells) limit the antitumor response. Consequently, both PD-L1 positive and negative tumors may be targeted using this approach. In humans, constitutive PD-L1 expression is normally limited to macrophage-lineage cells, although expression of PD-L1 can be induced on other hematologic cells as well, including activated T cells. However, aberrant expression of PD-L1 by tumor cells has been reported in a number of human malignancies. PD-L1 expressed by tumor cells has been shown to enhance apoptosis of activated tumor- specific T cells in vitro. Moreover, the expression of PD-L1 may protect the tumor cells from the inductionof apoptosis by effector T cells.

Nivolumab (BMS-936558; anti-PD-1 mAb) is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the programmed death-1 (PD-1) cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Blockade of the PD-1 pathway by nivolumab was studied using the mixed lymphocyte reaction (MLR). PD-1 blockade resulted in a reproducible enhancement of both proliferation and interferon release in the MLR. The effect of nivolumab on antigen-specific recall response was investigated using a CMV-restimulation assay with human peripheral blood mononuclear cells (PBMCs) and was evaluated by ELISA. Nivolumab monotherapy is currently approved for the treatment of advanced melanoma, lung cancer, metastatic renal and urothelial carcinoma, squamous cell carcinoma of the head and neck, hepatocellular carcinoma, MSI-H or dMMR metastatic colorectal cancer and Hodgkin lymphoma, and is being studied in several phase 3 and 2 clinical trials in advanced and metastatic solid and hematologic malignancies.

Ipilimumab is a fully humanized IgG1 monoclonal antibody binding to the anti-cytotoxic T-cell lymphoma-4 antigen (CTLA-4). Ipilimumab is an approved therapy for metastatic melanoma and has demonstrated improved overall survival as monotherapy and in combination with dacarbazine (11) (47) It has been studied in combination with multiple standards of care (SOC) therapies including chemotherapy for squamous and non-squamous NSCLC and radiotherapy for hormone resistant prostate cancer (48). Ipilimumab is currently also approved as adjuvant therapy in stage III melanoma.

Nivolumab in combination with ipilimumab is indicated for the treatment of patients with unresectable or metastatic melanoma and has been recently approved for the treatment of intermediate or poor risk advanced renal cell carcinoma, and MSI-H or dMMR metastatic colorectal cancer.

1.4.2 TMZ Mechanism of Action

Temozolomide is a triazene that undergoes rapid chemical conversion at physiologic pH to the active monomethyl triazenoimidazole carboxamide (MTIC). The cytotoxicity of MTIC is thought to be due primarily to alkylation at the O^6 position of guanine with additional alkylation also occurring at the N^7

position. Cytotoxic lesions that develop subsequently are thought to involve aberrant repair of the methyl adduct. Temozolomide is indicated for the treatment of patients with newly-diagnosed glioblastoma multiforme or malignant glioma, such as glioblastoma multiforme or anaplastic astrocytoma, showing recurrence or progression after standard therapy In mCRC, MGMT methylation has emerged as a potential biomarker of response to alkylating agents and several non-randomized clinical trials showed that the average ORR to TMZ is 10% in heavily pretreated patients (36) (38) (49) (50)

1.4.3 Summary of Results

Additional details are also available in the nivolumab, ipilimumab and TMZ Investigator Brochures (IB).

1.4.3.1 Summary of Safety

Ipilimumab Monotherapy

In study MDX010-20, the ipilimumab monotherapy arm was administered 3 mg/kg ipilimumab every 3 weeks for four doses. In this arm, there were 79% drug related adverse events, with 21% being Grade 3/4 and 3/131 (2%) Grade 5. The most frequent adverse events (AE) of interest were rash (30%), pruritus (33%), diarrhea (33%), colitis (8%), endocrine disorders (9%), AST/ALT increased (2%), and hepatitis (1%). Any grade immune related adverse events were 60% and the Grade 3/4 immune related adverse events for the same cohort were 13% with the most frequent adverse events being diarrhea (5%), colitis (5%), rash (2%), and endocrine disorders (3%). Comprehensive details on the safety profile of ipilimumab, including results from other clinical studies, are available in the ipilimumab product information and Investigator's Brochure.

Nivolumab Monotherapy

One study has contributed most to the clinical experience with nivolumab monotherapy in subjects with melanoma and other solid malignancies. CA209003 is an ongoing Phase 1 open label, multiple dose escalation study in 306 subjects with select previously treated advanced solid tumors, including melanoma, RCC, NSCLC, colorectal cancer, and hormone-refractory prostate cancer. Subjects received nivolumab at doses of 0.1, 0.3, 1, 3 or 10 mg/kg intravenously every 2 weeks, up to a maximum of 2 years of total therapy.As of 05-Mar-2013, a total of 107 melanoma subjects were treated with nivolumab in the dose range of 0.1- 10 mg/kg.

No maximal tolerated dose was identified in CA209003. The incidence, severity and relationship of AEs were generally similar across dose levels and tumor types. Nivolumab related AEs of any grade occurred in 72.4% of subjects. The most frequent nivolumab related AEs occurring in > 5% of subjects included: fatigue (25.7%), rash (13.5%), diarrhea (11.8%), pruritus (10.2%), nausea (7.9%), decreased appetite (7.9%), hemoglobin decreased (5.9%) and pyrexia (5.3%). The majority of events were low grade, with grade 3-4 drug related AEs observed in 14.8% of subjects. The most common Grade 3-4 drug-related AEs occurring in
> 1% of subjects were: fatigue (1.6%), lymphopenia (1.3%), abdominal pain (1%), diarrhea (1%), hypophosphatemia (1%) and pneumonitis (1%). At least one SAE was reported for 150 (49.3%) of the 304 subjects at all dose levels. Grade 3-4 SAEs were reported for 23 subjects (7.6%). Drug-related SAEs occurred in 11.5% of subjects. Grade 3-4 drug-related SAEs reported in at least 2 subjects included diarrhea (3 subjects, 1.0%), pneumonitis (3 subjects, 1.0%), pneumonia (2 subjects, 0.7%) and lipase increased (2 subjects, 0.7%). Additional select treatment-related AEs have occurred with low frequency (< 5%) but are considered clinically meaningful, as they require greater vigilance for early recognition and prompt intervention. These AEs include: ALT increased (4.3%), AST increased (3.6%), pneumonitis (3.3%), hypothyroidism (3.0%), hyperthyroidism (1.3%), renal failure (1.0%), adrenal insufficiency (0.7%) and colitis (0.7%). Grade 3-4 events of pneumonitis were reported in 3 subjects (1.0%) as described above (1 event was Grade 4). Grade 3 events of colitis, ALT increased, and AST increased were reported in 2 subjects(0.7%) each. Grade 3 events of adrenal insufficiency, hyperthyroidism, and hypothyroidism were reported in 1 subject (0.3%) each. Treatment-related AEs leading to discontinuation were reported in 18 (5.9%) of the

304 treated subjects in CA209003. The only events reported in more than 1 subject were pneumonitis (4 subjects; 1.3%) and hepatitis (2 subjects; 0.7%). There were 3 (1%) drug related deaths; each occurred after development of pneumonitis. Preliminary new non-clinical safety findings of adverse pregnancy outcomes and infant losses in the absence of overt maternal toxicity have been reported. The findings of increased late stage pregnancy loss and early infant deaths/euthanasia in nivolumab exposed pregnant monkeys suggest a potential risk to human pregnancy if there is continued treatment with nivolumab during pregnancy.
Additional details on the safety profile of nivolumab, including results from other clinical studies, are also available in the Investigator's Brochure.

Nivolumab Combined with Ipilimumab
In the Phase 1 study CA209004, ascending doses of nivolumab have been studied concomitantly with ascending doses of ipilimumab in subjects with unresectable or metastatic melanoma. In each arm in this multi-arm study, ipilimumab was administered once every 3 weeks for 4 doses with nivolumab administered once every 3 weeks for 8 doses. Starting at week 24, ipilimumab and nivolumab were administered once every 12 weeks for 8 doses. The three initial dose-escalation cohorts consisted of Cohort 1 (nivolumab 0.3 mg/kg plus ipilimumab 3 mg/kg; n = 14), Cohort 2 (nivolumab 1 mg/kg plus ipilimumab 3 mg/kg; n = 17) and Cohort 3 nivolumab 3 mg/kg plus ipilimumab 3 mg/kg; n = 6). Later, the study was amended to include Cohort 2a, which evaluated nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (n = 16). The following DLTs were observed in Cohort 1 - Grade 3 elevated AST/ALT (1 subject); in Cohort 2 - Grade 3 uveitis (1 subject) and Grade 3 elevated AST/ALT (1 subject) and in Cohort 3 - Grade 4 elevated lipase (2 subjects) and Grade3 elevated lipase (1 subject). Based on these data, Cohort 2 was identified as the maximum tolerated dose (MTD) and Cohort 3 exceeded the MTD.
As of 15-Feb-2013, a total of 53 melanoma subjects have been treated with nivolumab combined with ipilimumab in CA209004 across cohorts 1, 2, 2a, and 3, including subjects who received higher cumulative doses of the combination components than planned in the current study. At least one AE regardless of causality has been reported in 98% of subjects treated. The most common (reported at > 10% incidence) treatment-related AEs (any Grade %; Grade 3-4 %: 93; 53) are rash (55; 4), pruritus (47; 0), vitiligo (11; 0),
fatigue (38; 0), pyrexia (21, 0), diarrhea (34; 6), nausea (21, 0), vomiting (11, 2), ALT increased (21; 11), AST increased (21; 13), lipase increased (19; 13), amylase increased (15, 6), headache (11, 0), and cough (13, 0).
The majority of AEs leading to discontinuation (regardless of causality) were Grade 3 or 4 (reported in 11 of 53 subjects, 21%). Grade 3 events included lipase increased, ALT increased, AST increased, lipase increased, troponin I increased, colitis, diverticular perforation, pancreatitis, tachycardia, renal failure acute, choroiditis, autoimmune disorder, and pneumonitis. One subject each discontinued due to Grade 4 events of blood creatinine increased and AST increased. No drug-related deaths were reported.
Currently, the combination therapy of 3 mg/kg ipilimumab plus 1 mg/kg nivolumab is approved for the treatment of patients with BRAF V600 wild-type and BRAF V600 mutation-positive unresectable or metastatic melanoma.

CA209012 was a multi-arm Phase 1b trial evaluating the safety and tolerability of nivolumab in patients with chemotherapy-naïve advanced non-small cell lung cancer (NSCLC), as either a monotherapy or in combination with other agents including ipilimumab, at different doses and schedules. The primary endpoint of the study was safety with secondary endpoints of objective response rate (ORR) per RECIST 1.1 and 24- week progression-free survival (PFS). Participants were assigned to receive nivolumab 3 mg/kg Q2W + ipilimumab 1mg/kg Q12W (n=38), nivolumab 3mg/kg Q2W + ipilimumab 1 mg/kg Q6W (n=39) and nivolumab 3mg/kg Q2W (n=52). The confirmed ORR was 47% (N3 q2w + I1 q12w), 39% (N3 q2w + I1q6w) and 23% (N3 Q2W). The median duration of response (DOR) was not reached in any of these groups. The rate of treatment-related adverse events (AEs) in the Q12W (82%) and Q6W (72%) arms were comparable to monotherapy (72%). In the study, Grade 3/4 adverse events were 37%, 33%, and 19% for the

Q12W, Q6W, and nivolumab monotherapy arms, respectively. Treatment-related Grade 3-4 AEs led to discontinuation in 5% and 8% of participants in the Q12W and Q6W cohorts, respectively, and were similar to nivolumab monotherapy. There were no treatment-related deaths. The treatment-related select AEs in patients administered the optimized dosing schedule (3 mg/kg of nivolumab Q2W plus 1 mg/kg of ipilimumab Q6W) were skin related (36%), gastrointestinal (23%), endocrine (20%), and pulmonary (5%) and there were ≤ 5% treatment related Grade 3 and Grade 4 AEs per category (51).

The combination of nivolumab with ipilimumab is being studied in the Phase 1 study CA209016.

Subjects with metastatic RCC (mRCC) (Karnofsky performance status ≥ 80%; untreated or any number of prior therapies) were randomized to receive nivolumab 3 mg/kg + ipilimumab 1 mg/kg (arm N3 + I1) or nivolumab 1 mg/kg + ipilimumab 3 mg/kg (arm N1 + I3) IV Q3W for 4 doses followed by nivolumab 3 mg/kg IV Q2W until progression/toxicity. The primary objective was to assess safety/tolerability; secondary objective was to assess antitumor activity.

Subjects were randomized to N3 + I1 (n = 47) and N1 + I3 (n = 47). Approximately half (n = 46;51%) had prior systemic therapy (N3 + I1: 22; N1 + I3: 26).

After a median follow-up of 22.3 months, the confirmed ORR per RECIST 1.1 was 40.4% (N =47) in both Arms N3 + I1 and N1 + I3; 42.1% (n = 8) and 36.8% (n = 7) had an ongoing response, with a median DOR of 88.7 weeks (95% CI: 37.14, NA) and 85.9 weeks (95% CI: 35.14, NA), respectively. Median PFS was 7.7 months (95% CI: 3.71, 14.29) and 9.4 months (95% CI: 5.62,18.63) in Arms N3 + I1 and N1 + I3, respectively. OS at 12 months was 80.9% and 85.0% in Arms N3 + I1 and N1 + I3, respectively, and at 24 months was 67.3% and 69.6%, respectively. The safety of nivolumab combined with ipilimumab was assessed in study CA209016. Treatment-related AEs were seen in 88/94 pts (94%), including 43/47 (92%) inN3 + I1 and 45/47 (96%) in N1 + I3. The most frequently reported drug-related AEs in N3 + I1 included fatigue (66%), cough (53.2%), and arthralgia (51.1%); the majority were Grade 1-2. The most frequently reported drug-related AEs in N1 + I3 included fatigue (74.5%), nausea (55.3%), and diarrhea (53.2%). The majority were Grade 1-2. Treatment-related AEs leading to discontinuation (31.9% versus 10.6%), and treatment-related serious adverse events (SAEs) (34% versus 23.4%) occurred more commonly in subjects inthe N1 + I3 arm than in the N3 + I1 arm, respectively. (52) The combination of nivolumab and ipilimumab has demonstrated increased benefit compared to both ipilimumab monotherapy and nivolumab monotherapy. The deep anti-tumor response observed in study CA209004 is the basis for an ongoing randomized phase 3 study in advanced melanoma (CA209067). In Study CA209142, 55% of the participants with recurrent or metastatic MSI-H CRC treated with nivolumab 3 mg/kg combined with ipilimumab 1 mg/kg Q3W had objective response. Seventy-nine percent (79%) of participants achieved disease control for ≥ 12 weeks. Thisdurable and sustained response compares favorably to results with 3 mg/kg Q2W nivolumab monotherapy (Study CA209142).78 Studies investigating the efficacy and safety of nivolumab in combination withipilimumab are ongoing in NSCLC, RCC, and CRC. The combination of nivolumab and ipilimumab has the potential for increased frequencies of adverse events compared to ipilimumab monotherapy or nivolumab monotherapy. The most common (reported at > 10% incidence) treatment related AEs are fatigue, rash, pruritus, diarrhea, lipase increased, pyrexia, ALT increase, AST increased, amylase increased and vitiligo. This class of AEs are expected for thecombination of nivolumab and ipilimumab based on the known AE profile of each drug alone. In addition, many of the Grade 3-4 adverse events were laboratory in nature (i.e., liver function tests [LFTs], lipase, amylase), were without clinical sequelae and have been manageable and reversible following intervention dose delays or with systemic steroid treatment. However, these AEs have the potential to be fatal if not detected early and managed as per the established algorithm and fatal AEs have been reported for both ipilimumab and nivolumab monotherapy. Adverse drug reactions with fatal outcome in clinical trial

participants treated with nivolumab monotherapy and nivolumab and ipilimumab combination therapy are listed in the current version of the nivolumab IB (53).

TMZ

In patients treated with TMZ, whether used in combination with RT or as monotherapy following RT for newly-diagnosed glioblastoma multiforme, or as monotherapy in patients with recurrent or progressive glioma, the reported very common adverse reactions were similar: nausea, vomiting, constipation, anorexia, headache and fatigue. Convulsions were reported very commonly in the newly-diagnosed glioblastoma multiforme patients receiving monotherapy, and rash was reported very commonly in newly-diagnosed glioblastoma multiforme patients receiving TMZ concurrent with RT and also as monotherapy, and commonly in recurrent glioma. Most hematological adverse reactions were reported commonly or very commonly in both indications.

Newly-diagnosed glioblastoma multiforme

Myelosuppression (neutropenia and thrombocytopenia), which is a known dose-limiting toxicity for most cytotoxic agents, including TMZ, was observed. When laboratory abnormalities and adverse events were combined across concomitant and monotherapy treatment phases, Grade 3 or Grade 4 neutrophil abnormalities including neutropenic events were observed in 8 % of the patients. Grade 3 or Grade 4 thrombocyte abnormalities, including thrombocytopenic events were observed in 14 % of the patients who received TMZ.

Recurrent or progressive malignant glioma

In clinical trials, the most frequently occurring treatment-related undesirable effects were gastrointestinal disorders, specifically nausea (43 %) and vomiting (36 %). These reactions were usually Grade 1 or 2 (0 – 5 episodes of vomiting in 24 hours) and were either self-limiting or readily controlled with standard anti- emetic therapy. The incidence of severe nausea and vomiting was 4 %.

Grade 3 or 4 thrombocytopenia and neutropenia occurred in 19 % and 17 % respectively, of patients treated for malignant glioma. This led to hospitalization and/or discontinuation of TMZ in 8 % and 4 %, respectively. Myelosuppression was predictable (usually within the first few cycles, with the nadir between Day 21 and Day 28), and recovery was rapid, usually within 1-2 weeks. No evidence of cumulativemyelosuppression was observed. The presence of thrombocytopenia may increase the risk of bleeding, and the presence of neutropenia or leukopenia may increase the risk of infection.

In a population pharmacokinetics analysis of clinical trial experience there were 101 female and 169 male subjects for whom nadir neutrophil counts were available and 110 female and 174 male subjects for whom nadir platelet counts were available. There were higher rates of Grade 4 neutropenia (ANC < 0.5 x 109 /l), 12 % vs 5 %, and thrombocytopenia (< 20 x 109 /l), 9 % vs 3 %, in women vs men in the first cycle of therapy. In a 400 subject recurrent glioma data set, Grade 4 neutropenia occurred in 8 % of female vs 4 % of male subjects and Grade 4 thrombocytopenia in 8 % of female vs 3 % of male subjects in the first cycle of therapy. In a study of 288 subjects with newly-diagnosed glioblastoma multiforme, Grade 4 neutropenia occurred in 3 % of female vs 0 % of male subjects and Grade 4 thrombocytopenia in 1 % of female vs 0 % ofmale subjects in the first cycle of therapy.

1.5 Overall Risk/Benefit Assessment

Despite several therapies available for the treatment of mCRC, the incremental benefit of these treatments after treatment with a first line regimen is small and represents an area of unmet medical need. The safety profiles of all agents proposed for the combination are well defined and the products are already commercially available for the treatment of several advanced and metastatic tumor types. The safety profile of the combination of nivolumab with ipilimumab (3 mg/kg) is well characterized and is approved for the

treatment of unresectable or metastatic melanoma (54). The toxicity profile of the nivolumab + ipilimumab combination has been shown to correlate with the ipilimumab dose: with increasing doses of ipilimumab, there has been an increase in the frequency of adverse events and, potentially, the severity of these events; however, no novel toxicities have been demonstrated versus either agent alone. (55), (56), (57), (58), (59). In the current regimen for this protocol, the dose of ipilimumab will be cumulatively lower than the approved dose level for the combination for the treatment of advanced and metastatic melanoma. The toxicity profile with lower doses of ipilimumab has been established to be very similar to that of nivolumab monotherapy (60).

2 ETHICAL CONSIDERATIONS

2.1 Good Clinical Practice

This study will be conducted in full conformance with the International Conference on Harmonisation (ICH) E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting) and with the E.U. Clinical Trial Directive (2001/20/EC).

The study will be conducted in compliance with the protocol. The protocol and any amendments and the subject informed consent will receive Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion prior to initiation of the study. All potential serious breaches must be reported to the Sponsor immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.
Personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks.
This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment).

2.2 Institutional Review Board/Independent Ethics Committee

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevantsupporting information must be submitted to the IRB/EC by the Principal Investigator and reviewed and approved by the IRB/EC before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB/EC. The investigator should also provide the IRB/IEC with a copy of the Investigator Brochure or product labelling information to be provided to subjects and any updates.

Investigators are responsible for promptly informing the IRB/EC of any protocol amendments (see Section 11.4). In addition to the requirements for reporting all adverse events to the Sponsor, investigators must comply with requirements for reporting serious adverse events to the local health authority and IRB/EC.

2.3 Informed Consent

The Sponsor's sample Informed Consent Form will be provided to each site. Each Site can modify the template provided, but the Sponsor must review and approve any proposed deviations from the sample Informed Consent Forms or any alternate consent forms proposed by the site (collectively, the "Consent Forms") before IRB/EC submission. The final IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes according to local requirements.

Regarding specific Informed Consent(s) on optional tumor biopsies, the investigator or authorized designee will explain to each patient the objectives of the exploratory research. Patients will be told that they are free to refuse to participate and may withdraw their specimens at any time and for any reason.

Investigators must ensure that subjects are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate. In situations where consent cannot be given to subjects, their legally acceptable representatives are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which the subject volunteers to participate. The language of the consent must be non-technical and easily understood. Investigators should allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study. The Consent Forms must be signed and dated by the patient or the patient's legally authorized representative before his or her participation in the study. The case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study. A copy of each signed Consent Form must be provided to the patient or the patient's legally authorized representative. All signed and dated Consent Forms must remain in each patient's study file or in the site file and must be available for verification by study monitors at any time. If informed consent is initially given by a subject's legally acceptable representative or legal guardian, and the subject subsequently becomes capable of making and communicating his or her informed consent during the study, consent must additionally be obtained from the subject.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate. The final revised IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes.

Patients must be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and IRB/EC policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

2.4 Confidentiality

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient identification number. This means that patient names are not included in data sets that are transmitted to any Sponsor location.

Patient medical information obtained by this study is confidential and may only be disclosed to third parties as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law. Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes. Data generated by this study must be available for inspection upon request by representatives of the national and local health authorities, Sponsor's monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

2.5 Financial Disclosure

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study (i.e., last patient, last visit).

3 INVESTIGATIONAL PLAN

3.1 Study Design and duration

3.1.1 Overall study design

This is a Phase II, multicenter, single-arm trial designed to evaluate the efficacy and safety of TMZ plus NIVO and IPI combination in patients with metastatic MSS, MGMT silenced CRC.

After the Informed Consent signature, tumor specimens from candidate patients will be evaluated forsufficient amounts of viable tumor. Only patients whose tumors have sufficient amounts of viable tumor will be enrolled. Specimens will be centrally tested at the Pathology Department of the Sponsor for the concomitant presence of all the following:

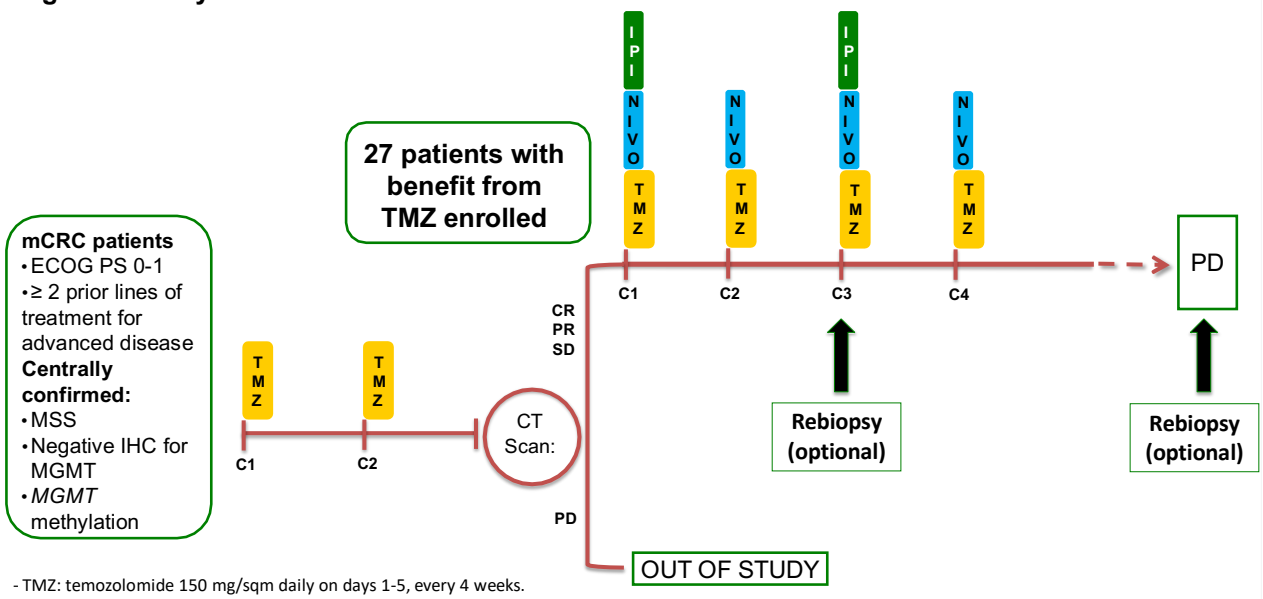
1. Confirmation of MGMT absent expression by IHC score.
2. Confirmation of MGMT methylation by pyrosequencing.
3. Confirmation of microsatellite stable (MSS) status according to multiplex polymerase chain reaction (PCR).

The following algorithm will be followed at the Sponsor: first, IHC for MGMT expression will be performed. Only in case of absent expression, will MGMT promoter methylation by pyrosequencing and mismatch-repair status according to multiplex PCR be performed.

Note: A separate tissue screening informed consent form shall be used to obtain consent to send the sample to the central laboratory. Subjects may continue on prior therapy while tissue testing takes place.

After central confirmation of eligibility (which will be provided by the Sponsor within 7 +/- 3 days from the arrival of tumor specimens), patients who meet the eligibility criteria will be enrolled across 10 Italian Centers. The study design is depicted in Figure 1.

Figure 1. Study Treatment



In the lead-in treatment phase, patients will be treated with:

- TMZ: 150 mg/sqm daily on days 1-5 every 4 weeks, for two cycles.

Temozolomide dose will be calculated according the following table:

150 mg/m / m ^{2} /day		**N. of 250 mg tablets at each dose**	**N. of 100 mg tablets at each dose**	**N. of 20 mg tablets at each dose**
Surface Area (m ^{2})	**Total Daily Dose (mg) ***	**Morning**	**Morning**	**Morning**
≤ 1.37	200	-	2	-
1.38 – 1.62	220	-	2	1
1.63 – 1.87	250	1	-	-
≥ 1.88	300	-	3	-

At the end of the first treatment phase, patients will undergo a CT scan at week 7 +/- 5 days, based on which:

- Patients with PD according to RECIST v1.1 will be out of the study.
- Twenty-seven patients with RECIST v1.1 CR/PR/SD will be evaluable in the ITT population and will enter the second treatment phase, being treated with the following NIVO+IPI+TMZ combination regimen:

  - NIVO: 480 mg i.v. every 4 weeks.
  - IPI: 1 mg/Kg i.v. every 8 weeks.
  - TMZ: 150 mg/sqm daily on days 1-5, every 4 weeks.

Treatment will continue until confirmed disease progression, unacceptable toxicity, withdrawal of consent or death, whichever occurs first.

- In case of unacceptable toxicity unequivocally due to TMZ, NIVO and IPI can be continued until disease progression, or to unacceptable toxicity, informed consent withdrawal or death.
- In case of unacceptable toxicity unequivocally due to NIVO and/or IPI, single-agent TMZ can be continued until disease progression, or to unacceptable toxicity, informed consent withdrawal or death.

In the second treatment phase, patients will undergo tumor assessment at baseline and every 8 +/- 1 weeks for the first 12 months following Cycle 1, Day 1. After 12 months, patients will undergo tumor assessments every 12 weeks until confirmed disease progression, unacceptable toxicity, withdrawal of consent or death, whichever occurs first.

Patients entering the second treatment phase can continue treatment after the first occurrence of unequivocal radiographic progression per RECIST v1.1, and until the criteria for progression according to ir-RECIST are met. After discussion with the Sponsor, patients will be permitted to continue study treatment after ir-RECIST criteria for progressive disease are met if they meet all of the following criteria:

- Evidence of clinical benefit (defined as the stabilization or improvement of disease-related symptoms) as assessed by the investigator
- Tolerance of study treatment
- Absence of symptoms and signs (including worsening of laboratory values; [e.g., new or worsening hypercalcemia]) indicating unequivocal progression of disease, despite a rising carcinoembryonic antigen (CEA) level
- No decline in ECOG performance status that can be attributed to disease progression
- Absence of tumor progression at critical anatomical sites (e.g., leptomeningeal disease) that cannot be managed by protocol−allowed medical interventions
- Patients must provide written consent to acknowledge deferring these treatment options in favor of continuing study treatment at the time of ir-RECIST - defined disease progression. All other elements of the main consent including description of reasonably foreseeable risks or discomforts, orother alternative treatment options will still apply.
- Sponsor approval

Patients who discontinue from study treatment for reasons other than disease progression (e.g., toxicity) will continue scheduled tumor assessments until disease progression, withdrawal of consent or death, whichever comes first. Patients who start a new anti-cancer therapy in the absence of disease progression should be followed according to the protocol schedule until there is confirmed disease progression, withdrawal of consent or death, whichever occurs first.

CD-ROM copies of the CT scans performed at baseline and during treatment until disease progression according to RECIST v1.1 and ir-RECIST criteria will be collected at the Coordinating Center (s.c. Oncologia Medica 1, Fondazione IRCCS Istituto Nazionale dei Tumori) for central review.

The sample of archived tumor tissues used for molecular screening, as well as blood and plasma samples, will be collected for exploratory biomarker assessments.

Patients will undergo an optional tumor biopsy sample collection, if clinically feasible as assessed by investigators, prior to C3D1 of the second treatment phase and/or at the evidence of radiographic disease progression causing treatment discontinuation. These data will be analyzed for the association between changes in tumor tissue and clinical outcome and to understand further the potential mechanisms of resistance to study treatment. A separate informed consent will be signed for optional biopsies requested by the study.

Longitudinal plasma samples (liquid biopsy and PBMCs) will be collected at baseline and every 4-week until best response according to ir-RECIST; after that, these samples (liquid biopsy and PBMCs) will be collected every 8 +/-1 weeks for the first 12 months and every 12 weeks thereafter and at disease progressionleading to treatment discontinuation.

Safety assessments will include the incidence, nature, and severity of adverse events, changes in vital signs and laboratory abnormalities graded per National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.0.

Quality of life will be assessed through Patient reported outcomes instruments (PRO) distributed by the study staff and completed in their entirety by the patient. PRO questionnaires should be completed every 8 weeks ± 7 days during treatment until PD, regardless of whether study treatment has been administered or held, and also if the patient is off treatment due to reason other than PD (i.e. refusal or unacceptable toxicity). After 12 months of treatment, PRO questionnaires will be completed every 12 weeks.

Post-progression, all patients will be followed for survival and subsequent anti-cancer therapy approximately every 3 months until death, loss to follow-up or withdrawal of consent, whichever occurs first.

3.1.2 Treatment beyond progression

Treatment beyond investigator-assessed RECIST 1.1-defined progression will be permitted if the subject experiences investigator-assessed clinical benefit and the subject is tolerating the study treatment (See Section 5.4).

3.1.3 Follow up

Follow-up begins when the decision to discontinue a subject from study therapy is made (no furthertreatment with study therapy).

- Subjects who discontinue treatment for reasons other than tumor progression will continue to have scheduled tumor imaging assessments (every 8 weeks +/- 1 week for the first 12 months following C1D1 and every 12 weeks thereafter) until the patient dies, experiences disease progression (investigator-assessed RECIST 1.1-defined progression), withdraws consent or until the study closes, whichever occurs first. Subjects who started a new anti-cancer therapy in the absence of disease progression should continue to be followed for progression according to the protocol schedule of response assessments unless consent is withdrawn or the patient experiences disease progression or death or until study termination, whichever occurs first.
- All adverse events will be recorded until 30 days after the last dose of study treatment, and SAEs and AESIs will be recorded until 90 days after last dose of study treatment. Ongoing adverse events thought to be related to study treatment will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, new anti-cancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or it has been determined that study treatment or participation is not the cause of the adverse event.
- Following early discontinuation or completion of the treatment period, all patients will be followed for survival and subsequent anti-cancer therapy beginning 3 months after the end of treatment visit. Survival and subsequent anti-cancer therapy follow-up information will be collected via telephone calls, patient medical records, and/or clinic visits approximately every 3 months until death, loss to follow-up, withdrawal of consent, or study termination by the Sponsor.

3.2 End of Study

The end of study is defined as the date of the last follow-up visit of the last patient enrolled and is expected to occur approximately 12 months after the last patient enrolls in the study. Follow-up for survival will continue until all patients have died or are lost to follow-up or the Sponsor decides to end the trial, whicheveroccurs first.

3.3 Data Monitoring Committee (DMC)

A Data Monitoring Committee (DMC) will be used to evaluate safety during the study. Safety data will be reviewed by the DMC on a periodic basis, approximately every 3 months from the date of first-patient-in. In addition, the DMC will review safety data 28 days after the inclusion of the 6th patient. Safety data, including demographics, adverse events, serious adverse events, and relevant laboratory data, will be reviewed.

The DMC will provide a recommendation as to whether the study may continue, whether amendment(s) to the protocol should be implemented, or whether the study should be stopped. The final decision will rest withthe Sponsor.

3.4 Scientific Rationale for study design

The aim of this trial is to evaluate the efficacy and safety of NIVO, IPI and TMZ combination in 27 patients with MSS, MGMT-silenced mCRC with initial clinical benefit following lead-in treatment with single-agent TMZ. The scientific background for this combination and this study design is extensively explained in Section 1.

3.4.1 Rationale for Blood Sampling for Biomarkers

Blood-based non-invasive biomarkers are emerging as an increasingly used tool in clinical practice. Our hypothesis is that we will find a relationship between tumor and plasma biomarkers (including but notlimited to PD-L1, PD-1, methyl-BEAMing, mutational load) and efficacy.

3.4.2 Rationale for the Collection of Tumor Specimens (Archival or Fresh)

An archival specimen, must be submitted as mandatory to the Sponsor. Since published results suggest that expression of PD-L1 in tumors correlates with response to anti−PD-1 therapy, tumor specimens fromenrolled patients will be tested for PD-L1 and PD-1 expression, as well as percentage of MGMT methylationby methyl-BEAMing and, in selected cases, mutational burden by whole exome sequencing.

Since prior therapies may have impacted immunohistochemical expression of MGMT, percentage of MGMT methylation (as assessed by quantitative techniques, such as digital PCR methods), and mutational burden, patients may submit specimens obtained after completion of their most recent prior therapy (optional).

In addition to assessment of PD-L1 status, other exploratory markers such as potential predictive and prognostic markers that are related to response or clinical benefit of TMZ, NIVO and IPI treatment, tumor immunobiology, mechanisms of resistance, or tumor type markers, may also be analyzed.

3.4.3 Rationale for the Collection of Tumor Specimens at the Time of Best response/Radiographic Progression

The requirement of a biopsy (if clinically feasible) to be performed at the time of best response, i.e. at C3D1 of the second treatment phase and/or disease progression is not only to confirm progression, but also to explore whether the tumor immune environment (e.g., tumor PD-L1, expression of other immune biomarkers) has changed since the beginning of the TMZ, NIVO and IPI treatment, which may help to investigate possible mechanisms of benefit and resistance to treatment.

3.4.4 Rationale for the Use of Modified Response Criteria (Immune Based)

Cancer immunotherapies may result in early apparent radiographic progression (pseudo progression/tumor immune infiltration), including the appearance of new lesions, followed by delayed response (61). Additionally, responding tumors may appear to increase in size because of the influx of immune cells (62) (63). Unconventional response patterns have been described in patients treated with anti−cytotoxic T lymphocyte−associated antigen 4 (CTLA-4) (61) and have been observed in the preliminary experience with ATEZO in Study PCD4989g. Therefore, ir-RECIST will be used in this study to accommodate the possible appearance of new lesions and to allow the apparent increase in tumor burden to be confirmed at a subsequent assessment prior to designation of progressive disease.

3.4.5 Rationale for Patient-Related Outcomes Assessments

The patient experience of mCRC includes impacts on symptoms (disease and treatment burden), functioning, and HRQoL. Disease-specific symptoms (e.g., bloating, abdominal pain, diarrhea, constipation, loss of appetite) and other symptoms (e.g., fatigue) that negatively impact patients' functioning and quality of life are relevant in this population. Patient-completed questionnaires are included to characterize the impact of

disease and treatment on patients. The collection of this type of data will provide greater insights for both the medical and patient communities to understand disease and treatment burden and resulting HRQoL when patients are exposed to TMZ, NIVO and IPI combination (64).

3.5 Outcome Measures

3.5.1 Efficacy Outcome Measures

The efficacy outcome measures for this study are as follows:

- Investigator-assessed PFS according to RECIST v1.1
- Investigator-assessed PFS according to ir-RECISTThe

secondary efficacy outcome measures are as follows:

- Investigator - assessed response rate per RECIST v1.1 or death from any cause on study
- Investigator - assessed response rate per ir-RECIST or death from any cause on study
- Investigator - assessed DOR per RECIST v1.1
- Investigator - assessed DOR per ir-RECIST
- OS
- Centrally-assessed objective response, DOR, PFS according to RECIST vers 1.1 and ir-RECIST

3.5.2 Safety Outcome Measures

The safety outcome measures for this study are as follows:

- Incidence, nature, and severity of adverse events, graded according to the NCI CTCAE v4.0
- Changes in vital signs, physical findings, and clinical laboratory results

A Data Monitoring Committee (DMC) will be used to evaluate safety during the study. Safety data will be reviewed by the DMC on a periodic basis, approximately every 3 months from the date of first-patient-in. In addition, the DMC will review safety data 28 days after the inclusion of the 6th patient. Safety data, including demographics, adverse events, serious adverse events, and relevant laboratory data, will be reviewed.

The DMC will provide a recommendation as to whether the study may continue, whether amendment(s) to the protocol should be implemented, or whether the study should be stopped. The final decision will rest withthe Sponsor.

3.5.3 Patient-Reported Outcome Measures

The PRO outcome measures for this study are as follows:

- EORTC QLQ-C30
- EORTC QLQ-CR29
- EuroQol EQ-5D

3.5.4 Exploratory Outcome Measures

The exploratory outcome measures for this study are as follows:

Longitudinal plasma samples (liquid biopsy and PBMCs) will be collected at baseline and every 4-week until best response according to ir-RECIST; after that, these samples (liquid biopsy and PBMCs) will be collected every 8 +/-1 weeks for the first 12 months and every 12 weeks thereafter and at disease progressionleading to treatment discontinuation. Patients will undergo an optional tumor biopsy sample collection, if

clinically feasible as assessed by investigators prior to C3D1 of the second treatment phase and/or at the evidence of radiographic disease progression causing treatment discontinuation. A separate informed consent will be signed for optional biopsies requested by the study.

Quantification of the percentage of MGMT methylation (by digital PCR based methods, i.e. methyl-BEAMing) and MGMT IHC will be performed in archival tumor tissue and tumor re-biopsies. Digital PCR for MGMT methylation status will be performed in cell-free circulating DNA (cfDNA) (cfDNA). Mutational load will be assessed in archival tumor tissues, tumor biopsies and cfDNA by means of whole exome sequencing. Immune-related tissue and circulating biomarkers will also be studied.

3.6 Post Study Access to Therapy

At the conclusion of the study, subjects who continue to demonstrate clinical benefit will be eligible to receive study drug. Study drug will be provided via an extension of the study, a rollover study requiring approval by responsible health authority and ethics committee or through another mechanism at the discretion of the Sponsor. The Sponsor reserves the right to terminate access to study drug if any of the following occur: a) the marketing application is rejected by responsible health authority; b) the study is terminated due to safety concerns; c) the subject can obtain medication from a government sponsored or private health program; or d) therapeutic alternatives become available in the local market.

4 STUDY POPULATION

The study is expected to screen approximately 400 patients to enroll approximately 100 patients who meet the eligibility criteria in the first lead-in treatment phase and 27 patients in the combination treatment phase.

4.1 Inclusion Criteria

Patients must meet the following criteria for study entry:

1. Have provided written informed consent prior to any study specific procedures
2. Willing and able to comply with the protocol.
3. ≥18 years of age.
4. ECOG status 0 – 1.
5. At least 12 weeks of life expectancy at time of entry into the study.
6. Histologically confirmed metastatic or inoperable adenocarcinoma of the colon and/or rectum, with centrally confirmed mismatch repair proficiency (microsatellite stable [MSS]) by multiplex polymerase chain reaction (PCR), MGMT promoter methylation by pyrosequencing and MGMT absent expression by IHC.
7. Patients with progressive disease or that are not candidate for oxaliplatin irinotecan fluoropyrimidine based chemotherapy and anti EGFR mAbs (in RAS/BRAF wild type tumors) in the metastatic setting.
8. Patients with documented disease relapsed within 6 months from the completion of adjuvant oxaliplatin-based chemotherapy are considered eligible.
9. Measurable, unresectable disease according to RECIST 1.1. Subjects with lesions in a previously irradiated field as the sole site of measurable disease will be permitted to enroll provided the lesion(s) have demonstrated clear progression and can be measured accurately.
10. Is willing and able to provide an adequate archival tumor sample (FFPE) available for tissuescreening for central tissue screening. If the tumor block is not available, a minimum of twentyfive 3-micron unstained sections on charged slides of tumor will be required.

4.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry:

1. Requirement for treatment with any medicinal product that contraindicates the use of any of the study medications, may interfere with the planned treatment, affects patient compliance or puts the patient at high risk for treatment-related complications.
2. Inability to swallow pills.
3. Refractory nausea and vomiting, malabsorption, external biliary shunt or significant bowel resection that would preclude adequate absorption.
4. Inadequate hematological function indicated by all of the following:
   – White Blood Cell (WBC) count < 2 x 10^9/L
   – Absolute neutrophil count (ANC) < 1.5 x 10^9/L
   – Platelet count < 100 x 10^9/L
   – Hemoglobin < 9 g/dL (patients may have transfusions and/or growth factors to attain adequate Hb)
5. Inadequate liver function indicated by all of the following:
   – Total bilirubin ≥ 1.5 x upper limit of normal (ULN)
   – Aspartate transaminase (AST) and alanine aminotransferase (ALT) ≥ 3 x ULN (≥ 5 x ULN in patients with known liver metastases)
   – Alkaline phosphatase (ALP) ≥ 2 x ULN (≥ 5 x ULN in patients with known liver metastases)
6. Inadequate renal function indicated by all of the following:
   – Serum creatinine > 1.5 x ULN or calculated creatinine clearance < 40 ml/min
7. INR > 1.5 and aPTT > 1.5 x ULN within 7 days prior to the start of study treatment for patients not receiving anti-coagulation.
   a. NOTE: The use of full-dose oral or parenteral anticoagulants is permitted as long as the INR or aPTT is within therapeutic limits (according to the medical standard of the enrolling institution) and the patient has been on a stable dose of anticoagulants for at least two weeks prior to the start of study treatment
8. Active infection requiring intravenous antibiotics at the start of study treatment.
9. Previous or concurrent malignancy, except for adequately treated basal or squamous cell skin cancer, superficial bladder cancer, or carcinoma *in situ* of the prostate, cervix, or breast, or other cancer for which the patient has been disease-free for three years prior to study entry.
10. Evidence of any other disease, neurologic or metabolic dysfunction, physical examination finding or laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of any of the study medications, puts the patient at higher risk for treatment-related complications or may affect the interpretation of study results.
11. Clinically significant (i.e. active) cardiovascular disease, for example cerebrovascular accidents ≤ 6 months prior to start of study treatment, myocardial infarction ≤ 6 months prior to study enrolment, unstable angina, New York Heart Association (NYHA) Functional Classification Grade II or greater congestive heart failure, or serious cardiac arrhythmia uncontrolled by medication or potentially interfering with protocol treatment.
12. History or evidence upon physical or neurological examination of central nervous system (CNS) disease (e.g. seizures) unrelated to cancer unless adequately treated with standard medical therapy.
13. Active brain metastases or leptomeningeal metastases. Subjects with brain metastases are eligible if these have been treated and there is no magnetic resonance imaging (MRI except where contraindicated in which CT scan is acceptable) evidence of progression for at least 8 weeks after treatment is complete and within 28 days prior to first dose of study drug administration. Cases should be discussed with the medical monitor. There must also be no requirement for

immunosuppressive doses of systemic corticosteroids (>10mg/day prednisone equivalents) for at least 2 weeks prior to study drug administration.

14. Surgical procedure (including open biopsy, surgical resection, wound revision, or any other major surgery involving entry into a body cavity) or significant traumatic injury within 28 days prior to start of study treatment, or anticipation of need for major surgical procedure during the course of the study.
15. Treatment with any chemotherapy, curative intent radiation therapy, biologics for cancer, or investigational therapy within 28 days of first administration of study treatment (subjects with prior cytotoxic or investigational products < 4 weeks prior to treatment might be eligible after discussion between investigator and sponsor, if toxicities from the prior treatment have been resolved to Grade 1 (NCI CTCAE version 4). Prior focal palliative radiotherapy must have been completed at least 2 weeks before study drug administration.
16. All toxicities attributed to prior anti-cancer therapy other than alopecia and fatigue must have resolved to Grade 1 (NCI CTCAE version 4) or baseline before administration of study drug. Subjects with toxicities attributed to prior anti-cancer therapy which are not expected to resolve and result in long lasting sequelae, such as neuropathy after platinum-based therapy, are permitted to enroll.
17. Known hypersensitivity to any of the study medications or Known hypersensitivity or allergy to Chinese hamster ovary cell products or any component of the NIVO formulation.
18. History of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins.
19. History of autoimmune disease including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Guillain-Barré syndrome, multiple sclerosis, vasculitis, or glomerulonephritis (see Appendix IV for a more comprehensive list of autoimmune diseases).
    a. Note: history of autoimmune-related hypothyroidism on a stable dose of thyroid replacement hormone may be eligible. Subjects with controlled type I diabetes mellitus on a stable insulin regimen, vitiligo or psoriasis not requiring systemic treatment may be eligible.
20. Prior allogeneic bone marrow transplantation or prior solid organ transplantation.
21. History of idiopathic pulmonary fibrosis (including pneumonitis), drug-induced pneumonitis, organizing pneumonia (i.e., bronchiolitis obliterans, cryptogenic organizing pneumonia), or evidence of active pneumonitis on Screening chest CT scan.
22. Treatment with systemic immunostimulatory agents (including but not limited to interferons or interleukin-2) within 4 weeks or five half-lives of the drug, whichever is shorter, prior to start of study treatment.
23. Treatment with systemic corticosteroids (>10 mg daily prednisone equivalents) or other systemic immunosuppressive medications (including but not limited to prednisone, dexamethasone, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-tumor necrosis factor [TNF] agents) within 2 weeks prior to start of study treatment, or requirement for systemic immunosuppressive medications during the trial. The use of inhaled corticosteroids and mineralocorticoids (e.g., fludrocortisone) is allowed.
    a. Note: Patients who have received acute, low-dose, systemic immunosuppressant medications (e.g., a one-time dose of dexamethasone for nausea) may be enrolled in the study after discussion with and approval by the Sponsor.
24. Positive test for human immunodeficiency virus (HIV).
25. Active hepatitis B (defined as having a positive hepatitis B surface antigen [HBsAg] test prior to randomization) or hepatitis C.

a. Note: Patients with past hepatitis B virus (HBV) infection or resolved HBV infection (defined as having a negative HBsAg test and a positive antibody to hepatitis B core antigen antibody test) are eligible. Patients with detectable HBV-DNA are not eligible.
b. Note: Patients positive for hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction testing is negative for HCV ribonucleic acid (RNA).

26. Active tuberculosis.
27. Administration of a live, attenuated vaccine within 4 weeks prior to start of study treatment or anticipation that such a live attenuated vaccine will be required during the study.
28. Prior treatment with CD137 agonists, anti-CTLA4, anti-PD-1, or anti-PD-L1 therapeutic antibody or drug specifically targeting T-cell co-stimulation or immune checkpoint pathways, including prior therapy with anti-tumor vaccines.
29. Pregnancy or lactation. A serum pregnancy test is required within 7 days prior to start of study treatment, or within 14 days with a confirmatory urine pregnancy test within 7 days prior start of study treatment.
30. For women who are not post-menopausal (< 12 months of non-therapy-induced amenorrhea) or surgically sterile (absence of ovaries and/or uterus): refusal to use a highly effective contraceptive method (i.e. with a failure rate of < 1% per year such as sexual abstinence, hormonal implants, combined oral contraceptives, vasectomized partner), during the study drug administration and for at least 6 months after the last dose of study medication. Periodic abstinence [e.g., calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable methods of contraception. A combination of male condom with cap, diaphragm or sponge with spermicide (double barrier methods) is not considered a highly effective birth control method. Acceptable methods of contraception may include total abstinence in cases where the lifestyle of the patient ensures compliance. A vasectomized partner is a highly effective birth control method provided that partner is the sole sexual partner of the trial participant and that the vasectomized partner has received medical assessment of the surgical success.
31. For men: refusal to use a highly effective contraceptive method (i.e. with a failure rate of < 1 % per year such as vasectomy, sexual abstinence or female partner use of hormonal implants or combined oral contraceptives) during the study drug administration and for a period of at least 6 months after the last dose of study medication. Periodic abstinence [e.g., calendar, ovulation, symptothermal, post ovulation methods] and withdrawal are not acceptable methods of contraception. A combination of male condom with either, cap, diaphragm or sponge with spermicide (double barrier methods) is not considered highly effective, birth control methods. Acceptable methods of contraception may include total abstinence in cases where the lifestyle of the patient ensures compliance. A vasectomized trial participant is a highly effective birth control method provided that the trial participant has received medical assessment of the surgical success.

4.3 Screen failures

Screen failures are defined as participants who consent to participate in the clinical study but who are not subsequently entered in the study/included in the analysis population. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, as applicable, and to respond to queries from regulatory authorities. Minimal information includes date of consent, demography, screen failure details, eligibility criteria, and any serious AEs.

5 TREATMENTS

5.1 Study treatments

An investigational product, also known as investigational medicinal product in some regions, is defined a pharmaceutical form of an active substance being tested or used as a reference in a clinical study, including products already with a marketing authorization but used or assembled (formulated or packaged) differently than the authorized form, or used for an unauthorized indication, or when used to gain further information about the authorized form.

Investigational products used in this trial are:

- TMZ
- Nivolumab, solution for injection
- Ipilimumab, solution for injection

TMZ will be used in commercially available formulation. Refer to prescribing information for details of the formulation as well as packaging and handling requirements. TMZ hard capsules (20 mg, 100 mg, and 250 mg) should be administered in the fasting state. The capsules must be swallowed whole with a glass of water and must not be opened or chewed.

Nivolumab (BMS-936558-01) will be with a potency of 100mg (10 mg/ml) and 40 mg (10 mg/mL).

Ipilimumab (BMS-734016) will be with a potency of 200 mg (5 mg/mL).

5.2 Treatment Administration

In the lead-in treatment phase, patients will be treated with:

- TMZ: 150 mg/sqm daily on days 1-5 every 4 weeks, for two cycles.

At the end of the first treatment phase, patients will undergo a CT scan at week 8 +/- 5 days, based on which:

- Patients with PD according to RECIST v1.1 will be out of study.
- Twenty-seven patients with RECIST v1.1 CR/PR/SD will be evaluable in the ITT population and will enter the second treatment phase, being treated with the following NIVO+IPI+TMZ combination regimen:
  - NIVO: 480 mg i.v. every 4 weeks.
  - IPI: 1 mg/Kg i.v. every 8 weeks.
  - TMZ: 150 mg/sqm daily on days 1-5, every 4 weeks.

Treatment will continue until confirmed disease progression, unacceptable toxicity, withdrawal of consent or death, whichever occurs first.

- In case of unacceptable toxicity unequivocally due to TMZ, NIVO and IPI can be continued until disease progression, or to unacceptable toxicity, informed consent withdrawal or death.
- In case of unacceptable toxicity unequivocally due to NIVO and/or IPI, single-agent TMZ can be continued until disease progression, or to unacceptable toxicity, informed consent withdrawal or death.

5.3 Dosage Modification

5.3.1 Dose Delay Criteria

Reasons for dose modifications or delays, the supportive measures taken, and the outcome will be documented in the patient's chart and recorded in the eCRF.

For toxicities which are considered by the investigator unlikely to develop into serious or life-threatening events (e.g. alopecia, altered taste etc.), treatment will be continued at the same dose without reduction or interruption. In addition, no dose reductions or interruptions will be required for anemia (non-hemolytic) as it can be satisfactorily managed by transfusions or erythropoietin. Where several toxicities with different grades or severity occur at the same time, the dose modifications applied should be the greatest reduction applicable.

If, in the opinion of the Investigator, a toxicity is considered to be due solely to one drug (e.g. immune- related adverse events related to IPI and/or NIVO), the dose of the other drugs does not require modification.

If a delay related to a study drug alone is required, the whole study treatment should be delayed as well until the requirements for restarting the therapy are met.

Dose modifications for isolated abnormal hematologic lab values will be based on hematological parameters at start of a treatment cycle.

Nivolumab or ipilimumab administration should be delayed for the following:

- Grade 2 non-skin, drug-related adverse event, with the exception of fatigue
- Grade 2 drug-related creatinine, AST, ALT, and/or Total Bilirubin abnormalities
- Grade 3 skin, drug-related adverse event
- Grade 3 drug-related laboratory abnormality, with the following exceptions: – Grade 3 lymphopenia or asymptomatic amylase or lipase does not require dose delay – Grade ≥ 3 AST, ALT, Total Bilirubin will require dose discontinuation (see Section 6.1)
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

Participants who require delay of nivolumab/ipilimumab should be re-evaluated weekly or more frequently if clinically indicated and resume nivolumab/ipilimumab dosing when re-treatment criteria are met.

In case of unacceptable toxicity unequivocally due to TMZ, NIVO and IPI can be continued until disease progression, or to unacceptable toxicity, informed consent withdrawal or death.

In case of unacceptable toxicity unequivocally due to NIVO and/or IPI, single-agent TMZ can be continued until disease progression, or to unacceptable toxicity, informed consent withdrawal or death.

5.3.2 Criteria to Resume Treatment with Nivolumab and/or Ipilimumab

Participants may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤ 1 or baseline value, with the following exceptions:

- Participants may resume treatment in the presence of Grade 2 fatigue
- Participants who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- For participants with Grade 2 AST, ALT, or Total Bilirubin elevations, dosing may resume when laboratory values return to baseline and management with corticosteroids, if needed, is complete.
- Drug-related pulmonary toxicity, diarrhea or colitis must have resolved to baseline before treatment is resumed. Participants with persistent Grade 1 pneumonitis after completion of a steroid taper over at least 1 month may be eligible for retreatment if discussed with and approved by Sponsor.
- Participants with drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment after consultation with the Sponsor Medical Monitor (ordesignee). Adrenal insufficiency requires discontinuation regardless of control with hormone replacement

5.3.3 Treatment of Nivolumab- or Ipilimumab-related Infusion Reactions

Since nivolumab and ipilimumab contain only human immunoglobulin protein sequences, they are unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to 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 study medical monitor/designee and reported as an SAE if it meets the criteria. Infusion reactions should be graded according to NCI CTCAE (current version) guidelines. 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 participant until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg at least 30 minutes before additional nivolumab administrations.

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

- Stop the study drug infusion, begin an IV infusion of normal saline, and treat the participant with diphenhydramine 50 mg IV (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg; remain at bedside and monitor participant 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 participant closely. If symptoms recur, then no further study medication 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 should be administered at least 30 minutes before nivolumab and/or ipilimumab infusions. If necessary, corticosteroids (up to 25 mg of hydrocortisone or equivalent) may be used.

For **Grade 3 or 4** symptoms: (severe reaction, Grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates). Grade 4: Life threatening; pressor or ventilatory support indicated):

- Immediately discontinue infusion of study drug. Begin an IV infusion of normal saline and treat the participant 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. Participant should be monitored until the Investigator is comfortable that the symptoms will not recur. Study drug will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor participant until recovery of the 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).

5.3.4 Management Algorithms for Immuno-Oncology Agents

Details on immune related adverse event management algorithm can be found in Appendix VIII for the following categories:

- GI Adverse Event
- Renal Adverse Event
- Pulmonary Adverse Event
- Hepatic Adverse Event
- Endocrinopathy Management
- Skin Adverse Event
- Neurological Adverse Event

5.3.5 Dose Reductions for TMZ

Dose modifications criteria for TMZ-related adverse events are listed below:

TMZ dose levels		
Dose level	TMZ dose (mg/mq/day)	Remarks
0	150	Dose during Cycle 1
- 1	113	Due to prior toxicity
- 2	75	Due to prior toxicity
TMZ dose reduction		
Toxicity	Reduce TMZ by 1 dose level	Discontinue TMZ
Absolute neutrophil count	$<1.0 \times 10^9$/l	If dose level -1 still results in unacceptable toxicity
Thrombocyte count	$<50 \times 10^9$/l	
CTC non-hematological Toxicity (except for alopecia, nausea, vomiting)	CTC grade 3	CTC grade 4

For dose calculations, please refer to the following Table:

25% dose reduction = 113 mg/m / m ^{2} /day		**N. of 250 mg tablets at each dose**	**N. of 100 mg tablets at each dose**	**N. of 20 mg tablets at each dose**
Surface Area (m ^{2})	**Total Daily Dose (mg) ***	**Morning**	**Morning**	**Morning**
≤ 1.37	160	-	1	3
1.38 – 1.62	180	-	1	4
1.63 – 1.87	200	-	2	-
≥ 1.88	220	-	2	1
50% dose reduction 75 mg/m / m ^{2} / day		**N. of 250 mg tablets at each dose**	**N. of 100 mg tablets at each dose**	**N. of 20 mg tablets at each dose**
Surface Area (m ^{2})	**Total Daily Dose (mg) ***	**Morning**	**Morning**	**Morning**
≤ 1.37	100	-	1	-
1.38 – 1.62	120	-	1	1
1.63 – 1.87	140	-	1	2
≥ 1.88	160	-	1	3

5.4 Treatment Beyond Disease Progression

Accumulating evidence indicates a minority of participants treated with immunotherapy may derive clinical benefit despite initial evidence of PD.

Dosing of study treatment beyond ir-RECIST−defined disease progression is allowed for patients. Patients must meet all of the following criteria to be allowed to receive study treatment beyond disease progression:

- Evidence of clinical benefit (defined as the stabilization or improvement of disease-related symptoms) as assessed by the investigator
- Tolerance of study treatment
- Absence of symptoms and signs (including worsening of laboratory values; [e.g., new or worsening hypercalcemia]) indicating unequivocal progression of disease, despite a rising CEA level
- No decline in ECOG performance status that can be attributed to disease progression
- Absence of tumor progression at critical anatomical sites (e.g., leptomeningeal disease) that cannot be managed by protocol−allowed medical interventions
- Patients must provide written consent to acknowledge deferring other treatment options in favor of continuing study treatment at the time of ir-RECIST - defined disease progression. All other

elements of the main consent including description of reasonably foreseeable risks or discomforts, or other alternative treatment options will still apply.

- Sponsor approval

Patients in whom radiographic disease progression is confirmed at a subsequent tumor assessment (within 6 weeks) may be considered for continued study treatment at the discretion of the investigator if they continue to meet the criteria above and have evidence of clinical benefit. The assessment of clinical benefit should be balanced by clinical judgment as to whether the participant is clinically deteriorating and unlikely to receive any benefit from continued treatment with study treatment.

5.5 Preparation/Handling/Storage/Accountability

The investigational products should be stored in a secure area according to local regulations. It is the responsibility of the investigator to ensure that investigational products are only dispensed to study Participants. The investigational products must be dispensed only from official study sites by authorized personnel according to local regulations. Vials of nivolumab injection must be stored at 2°C to 8°C (36°F to 46°F) and protected from light and freezing. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours. The administration of nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored under refrigeration conditions (2°C to 8°C, 36°F to 46°F) for up to 24 hours, and a maximum of 8 hours of the total24 hours can be at room temperature (up to 25°C, 77°F) and room light. The maximum of 8 hours under room temperature and room light conditions includes the product administration period.

Ipilimumab injection, 50 mg/10 mL (5 mg/mL) or 200 mg/40 mL (5 mg/mL), must be stored refrigerated (2°C to 8°C) and protected from light. Ipilimumab injection must not be frozen. Partially used vials or emptyvials of ipilimumab injection should be discarded at the site according to appropriate drug disposal procedures.

Ipilimumab injection may be stored undiluted (5 mg/mL) or following dilution in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP in PVC, non-PVC/non-DEHP, or glass containers for up to 24 hours at 2°C to 8°C or room temperature/room light.

Recommended safety measures for preparation and handling include protective clothing, gloves, and safety cabinets.

If concerns regarding the quality or appearance of the study treatment arise, the study treatment should notbe dispensed and contact the Sponsor immediately. Study treatment not supplied by the Sponsor will be stored in accordance with the package insert and IB: Temozolomide will be stored at room temperature (upto 25°C, 77°F). Investigational product documentation (whether supplied by the Sponsor or not) must be maintained that includes all processes required to ensure drug is accurately administered. This includes documentation of drug storage, administration and, as applicable, storage temperatures, reconstitution, and use of required processes (e.g., required diluents, administration sets).

5.6 Concomitant Treatments

5.6.1 Permitted Treatments

Concomitant therapy includes any prescription medications or over-the-counter preparations used by apatient between the 7 days preceding the screening evaluation and the treatment discontinuation visit. Any concomitant therapy administered during this period should be recorded on the appropriate eCRF.

Patients who experience infusion-associated symptoms may be treated symptomatically with acetaminophen, ibuprofen, diphenhydramine, and/or famotidine or another H2 receptor antagonist, as per standard practice. Serious infusion-associated events manifested by dyspnea, hypotension, wheezing, bronchospasm,

tachycardia, reduced oxygen saturation, or respiratory distress should be managed with supportive therapies as clinically indicated (e.g., supplemental oxygen and β2-adrenergic agonists).

Participants are permitted the use of topical, ocular, intra-articular, intranasal and inhalation corticosteroids (with minimal system absorption).

Adrenal replacement steroid doses > 10 mg daily prednisone are permitted. A brief (less than 3 weeks) course of corticosteroids for prophylaxis (e.g., for contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.

Premedication for NIVO and IPI may be administered for Cycles ≥ 2 at the discretion of the treating physician after consultation with the Sponsor.

The use of inhaled corticosteroids and mineralocorticoids (e.g., fludrocortisone) for patients with orthostatic hypotension or adrenocortical insufficiency is allowed. Megestrol administered as an appetite stimulant is acceptable while the patient is enrolled in the study.

Influenza vaccination should be given during influenza season only (approximately October to March). Patients must not receive live, attenuated influenza vaccine within 4 weeks prior to Cycle 1, Day 1 or at any time during the study but may receive inactivated vaccines.

Patients who use hormonal therapy with oral contraceptives, hormone-replacement therapy, prophylactic or therapeutic anticoagulation therapy (such as low−molecular weight heparin or warfarin at a stable dose level), or other allowed therapy should continue their use.

Males and females of reproductive potential should use highly effective means of contraception.

Prior palliative radiotherapy must have been completed at least 2 weeks prior to first dose.

5.6.2 Prohibited and/or Restricted Treatments

The following medications are prohibited during the study (unless utilized to treat a drug-related adverse event). Medications taken within 2 weeks prior to initial study drug administration and medications taken during study must be recorded on the CRF.

- Immunosuppressive agents, including but not limited to cyclophosphamide, azathioprine, methotrexate, and thalidomide.
- Immunosuppressive doses of systemic corticosteroids (except as stated in Section 5.6.1)
- Any concurrent antineoplastic therapy (i.e., chemotherapy, hormonal therapy, immunotherapy, extensive, non-palliative radiation therapy, or standard or investigational agents for treatment of cancer)
- Any botanical preparation (e.g. herbal supplements or traditional Chinese medicines) intended totreat the disease under study or provide supportive care. Use of marijuana and its derivatives for treatment of symptoms related to cancer or cancer treatment are permitted if obtained by medical prescription or if its use (even without a medical prescription) has been legalized locally.
- Any live / attenuated vaccine (e.g. varicella, zoster, yellow fever, rotavirus, oral polio and measles, mumps, rubella (MMR)) during treatment and until 100 days post last dose.
- Initiation or increased dose of granulocyte colony-stimulating factors (e.g., granulocyte colony-stimulating factor, granulocyte/macrophage colony-stimulating factor, and/or pegfilgrastim) is strongly discouraged.
- Patients are not allowed to receive immunostimulatory agents, including but not limited to IFN-α, IFN-γ, or IL-2, during the entire study.
- All patients (including those who discontinue the study early) should not receive other immunostimulatory agents for 10 weeks after the last dose of NIVO and IPI.

Participants with a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of treatment assignment are excluded. Inhaled or topical steroids, and adrenal replacement steroid doses > 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease.

Any concomitant therapy intended for the treatment of cancer, whether health authority approved or experimental, is prohibited during the study period. This includes but is not limited to the following:

- Chemotherapy, hormonal therapy, immunotherapy, radiotherapy, investigational agents, or herbal therapy

After the completion of Cycle 1, palliative radiotherapy may be considered if patients are deriving benefit (e.g., treatment of known bone metastases).

Patients experiencing a mixed response requiring local therapy (e.g., surgery, stereotactic radiosurgery, radiotherapy, radiofrequency ablation) for control of three or fewer lesions may still be eligible to continue study treatment, after the approval of the Sponsor. Patients who receive local therapy directed at a target lesion will no longer be evaluable for radiographic response but will remain evaluable for progression.

5.7 Treatment Compliance

Study treatment compliance will be periodically monitored by drug accountability. Drug accountability should be reviewed by the site study staff at each visit to confirm treatment compliance. Sites should discuss discrepancies with the participant at each on-treatment study visit.

6 TREATMENT DISCONTINUATION CRITERIA

6.1 Discontinuation from Study Treatment

Patients must discontinue study treatment if they experience any of the following:

- Symptomatic deterioration (i.e., uncontrollable pain secondary to disease or unmanageable ascites, etc.) attributed to disease progression as determined by the investigator after integrated assessment of radiographic data, biopsy results, and clinical status
- Intolerable toxicity related to TMZ, NIVO and IPI including development of an immune-mediated adverse event determined by the investigator to be unacceptable given the individual patient's potential response to therapy and severity of the event
- Any medical condition that may jeopardize the patient's safety if he or she continues on study treatment
- Use of another non-protocol anti-cancer therapy
- Pregnancy

6.2 Discontinuation from the Study

Patients have the right to voluntarily withdraw from the study at any time for any reason. In addition, the investigator has the right to withdraw a patient from the study at any time. Reasons for withdrawal from the study may include but are not limited to the following:

- Patient withdrawal of consent at any time
- Any medical condition that the investigator or Sponsor determines may jeopardize the patient's safety if he or she continues in the study
- Investigator or Sponsor determines it is in the best interest of the patient

- Patient non-compliance

Every effort should be made to obtain information on patients who withdraw from the study.

The withdrawal of consent should be explained in detail in the medical records by the investigator, as to whether the withdrawal is from further treatment with study treatment only or also from study procedures and/or post treatment study follow-up and entered on the appropriate CRF page. However, patients will not be followed for any reason after consent has been withdrawn.

6.3 Post Study Drug Study Follow up

Following early discontinuation or completion of the treatment period, all patients will be followed for survival and subsequent anti-cancer therapy beginning 3 months after the end of treatment visit. Survival andsubsequent anti-cancer therapy follow-up information will be collected via telephone calls, patient medical records, and/or clinic visits approximately every 3 months until death, loss to follow-up, withdrawal of consent, or study termination by the Sponsor.

The investigator should follow each adverse event until the event has resolved to baseline grade or better, the event is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all serious adverse events considered related to study drug or trial-related procedures until a final outcome can be reported.

During the study period, resolution of adverse events (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification.

All pregnancies reported during the study should be followed until pregnancy outcome. If the electronic data capture (EDC) system is not available at the time of pregnancy outcome, follow reporting instructions provided in Section 8.7.

For serious adverse events, non-serious adverse events of special interest, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

6.4 Withdrawal of Consent

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. However, patients will not be followed for any reason after consent has been withdrawn.

6.4.1 Withdrawal from the exploratory studies

Patients who give consent to provide specimens have the right to withdraw their specimens from the exploratory studies at any time for any reason. If a patient wishes to withdraw consent to the testing of his or her specimens, the investigator must inform the Sponsor in writing of the patient's wishes. The patient will be provided with instructions on how to withdraw consent after the trial is closed. A patient's withdrawal from the main Study does not, by itself, constitute withdrawal of specimens from the exploratory studies. Likewise, a patient's withdrawal from the exploratory studies does not constitute withdrawal from the main study.

6.5 Lost to Follow-Up

All reasonable efforts must be made to locate participants to determine and report their ongoing status. This includes follow-up with persons authorized by the participant.

Lost to follow-up is defined by the inability to reach the participant after a minimum of three documented phone calls, faxes, or emails as well as lack of response by participant to one registered mail letter. All attempts should be documented in the participant's medical records.

If it is determined that the participant has died, the site will use permissible local methods to obtain date and cause of death.

If investigator's use of third-party representative to assist in the follow-up portion of the study has been included in the participant's informed consent, then the investigator may use a Sponsor retained third-party representative to assist site staff with obtaining participant's contact information or other public vital status data necessary to complete the follow-up portion of the study.

The site staff and representative will consult publicly available sources such as public health registries and databases to obtain updated contact information.

If after all attempts the participant remains lost to follow-up, then the last known alive date as determined by the investigator should be reported and documented in the participant's medical records.

6.6 Study and Site Discontinuation

The Sponsor has the right to close a site at any time. Reasons for closing a site may include but are not limited to the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice
- No study activity (i.e., all patients have completed, and all obligations have been fulfilled).

The Sponsor will notify the investigator if the Sponsor decides to discontinue the study.

7 STUDY ASSESSMENTS AND PROCEDURES

All the study assessments are described in the schedule of assessment (Appendix I). Protocol waivers or exemptions are not allowed. Adherence to the study design requirements, including those specified in the Schedule of Activities, is essential and required for study conduct.

All immediate safety concerns must be discussed with the Sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue treatment.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria before treatment assignment/randomization. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (e.g., blood count) andobtained before signing of informed consent may be utilized for screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed within the timeframe defined in the Schedule of Activities. Additional measures, including non-study required laboratory tests, should be performed as clinically indicated or to comply with local regulations. Laboratory toxicities (e.g., suspected drug induced liver enzyme evaluations) will be monitored during the follow-up phase via on site/local labs until all study drug related toxicities resolve, return to baseline, or are deemed irreversible.

If a participant shows pulmonary-related signs (hypoxia, fever) or symptoms (e.g. dyspnea, cough, fever) consistent with possible pulmonary adverse events, the participant should be immediately evaluated to rule out pulmonary toxicity, according to the suspected pulmonary toxicity management algorithm in the BMS- 936558 (nivolumab) Investigator's Brochure.

Some of the assessments referred to in this section may not be captured as data in the eCRF. They are intended to be used as safety monitoring by the treating physician. Additional testing or assessments may be performed as clinically necessary or where required by institutional or local regulations.

7.1 Assessments during Treatment

All visits must occur within ± 3 days from the scheduled date unless otherwise noted (see Appendix I). All assessments will be performed on the day of the specified visit unless a time window is specified. Assessments scheduled on the day of study treatment administration (Day 1) of each cycle should be performed prior to study treatment infusion unless otherwise noted.

See the study flowchart provided in Appendix I for the schedule of treatment period assessments.

The following assessments may be performed ≤ 48 hours before Day 1 of each cycle: ECOG performance status, limited physical examination, laboratory tests, adverse event evaluation, and concomitant medication evaluation.

Blood samples for exploratory research will be obtained according to the schedule in Appendix I (at every day 1 of each cycle until best response according to ir-RECIST, then every 8 +/- 1 weeks for the first 12 months and every 12 weeks thereafter and at disease progression leading to treatment discontinuation). See the laboratory manual for additional details on blood sample handling.

Optional biopsy with collection of fresh-frozen tumor tissue and FFPE tumor tissue (see laboratory manual for details of tissue collection and handling) may be collected per investigator discretion and only in consenting patients, prior to C3D1 of the second treatment phase and/or at the time of radiographic progression leading to treatment discontinuation.

7.2 Informed Consent Forms and Screening Log

Written informed consent for participation in the study must be obtained before performing any study- specific screening tests or evaluations and may be obtained up to 28 days before initiation of study treatment.

Informed Consent Forms for enrolled patients and for patients who are not subsequently enrolled will be maintained at the study site.

Note: A separate tissue screening informed consent form may be used to obtain consent to send the sample to the central laboratory. Subjects may continue on prior therapy while tissue testing takes place.

All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before enrolment. The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

7.3 Medical History and Demographic Data

Medical history and demographic data should be recorded during the screening period (-28 to -1 days). Medical history includes clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, and all medications (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within

7 days prior to the screening visit. A history of pleural or pericardial effusion or of ascites requiring intervention should be entered in the medical history.

Demographic data will include age, ECOG performance status, sex, and self-reported race/ethnicity.

7.4 Physical Examinations

A complete physical examination should include an evaluation of the head, eyes, ears, nose, and throat and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. Any abnormality identified at baseline prior to the first treatment phase should be recorded on the General Medical History and Baseline Conditions eCRF. Height and weight should be measured and recorded in the eCRF. Weight should be measured and recorded at the beginning of each cycle.

If physical examinations are assessed within 7 days of the Cycle 1 Day 1 visit, they do not have to be repeated at Day 1.

At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

Eastern Cooperative Oncology Group Performance Status (ECOG PS) should be assessed before each infusion.

7.5 Vital Signs

Vital signs will include measurements of pulse rate, respiratory rate, blood oxygen saturation, systolic and diastolic blood pressures while the patient is in a seated position, and temperature. They should be determined and recorded at screening and at every subsequent visit.

For the first infusion, the patient's vital signs (pulse rate, respiratory rate, blood pressure, and temperature) should be determined within 60 minutes before and 30 (± 10) minutes after the infusion. During the infusion, the patient's vital signs will be determined only in presence of distress/infusion-related reactions. For subsequent infusions, vital signs will be collected within 60 minutes before infusion and at the end of the infusion. Patients will be informed about the possibility of delayed post-infusion symptoms and instructed to contact their study physician if they develop such symptoms.

7.6 Efficacy assessments

7.6.1 Tumor and Response Evaluations

Screening assessments with CT scans (with contrast unless contraindicated) of the chest, abdomen, and pelvis are preferred; should a patient have a contraindication for CT IV contrast, a non-contrast CT of the chest and a contrast enhanced MRI of the abdomen and pelvis and other known/suspect sites of disease may be obtained. Should a participant have contraindication for both MRI and CT intravenous contrasts, a non- contrast CT of the chest and a non-contrast MRI of the abdomen, pelvis, and other known/suspected sites of disease should be obtained.

A CT (with contrast if not contraindicated) or MRI scan of the brain must be done at screening to exclude CNS metastases. An MRI scan of the brain is required to confirm or refute the diagnosis of CNS metastases at baseline in the event of an equivocal scan. Patients with definitively treated stable CNS metastases may be eligible for the study (see Section 4.2).

PET alone will not be considered for the disease assessment. Complementary CT and/or MRI or biopsy must be performed in such cases. Note: Use of CT component of a PET/CT scanner: Combined modality scanning, such as with FDG-PET/CT, is increasingly used in clinical care and is a modality/technology thatis in rapid evolution; therefore, the recommendations outlined here may change rather quickly with time. At present, low-dose or attenuation correction CT portions of a combined FDG-PET/CT are of limited use in anatomically-based efficacy assessments, and it is, therefore, suggested that they should not be substitutedfor dedicated diagnostic contrast enhanced CT scans for anatomically-based RECIST 1.1 (Appendix III) measurements. However, if a site can document that the CT performed as part of a FDG PET/CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the FDG- PET/CT can be used for RECIST 1.1 measurements. Note, however, that the FDG-PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Results of standard of care tests or examinations performed prior to obtaining Informed Consent and 28 to 1 day prior to study entry may be used for the purposes of Screening rather than repeating such tests.

Evaluation of tumor response conforming to RECIST v1.1 and ir-RECIST must be documented every8 weeks ± 1 week no matter where the patient is in the study treatment cycle (this can occur mid-treatment) until documented investigator−determined progressive disease, loss of clinical benefit, withdrawal of consentor death, whichever occurs first. After 12 months, patients will undergo tumor assessments every 12 weeks until confirmed disease progression.

The same radiographic procedure used to assess disease sites at screening should be used throughout the study (e.g., the same contrast protocol for CT scans). All known sites of disease must be documented at screening and reassessed at each subsequent tumor evaluation. Response will be assessed by the investigator using ir-RECIST and RECIST v1.1 (see Appendix II and Appendix III).

At the investigator's discretion, CT scans should be repeated at any time if progressive disease is suspected.

Per RECIST 1.1, partial or complete response should be confirmed by a repeat tumor imaging assessment, preferably at the next scheduled imaging visit, and not less than 4 weeks from the date the response was first documented.

Scheduled tumor assessments are independent of any changes to the study treatment administration schedule (e.g., dose delay) and may occur mid-cycle depending on length of cycle. If a tumor assessment has to be performed early or late, subsequent assessments should be conducted according to the original schedule based on the date of first study drug administration (Cycle 1 Day 1). Patients who discontinue study treatment for any reason other than disease progression will continue to undergo tumor response evaluations (every 8 weeks and every 12 weeks after 12 months) until progressive disease. A rising CEA alone without radiological evidence of progression is not considered progressive disease. Patients who continue to experience clinical benefit, despite evidence of radiographic progression as defined by ir-RECIST, may continue treatment (see Section 5.4) and will continue tumor assessments as per the schedule listed above.

CD-ROM copies of the CT scans performed according to treatment will be collected at the Coordinating Center (s.c. Oncologia Medica 1 Fondazione IRCCS Istituto Nazionale dei Tumori) for central review.

Site should follow their local privacy practices to de-identify all subject identifying information (name, medical record number, etc.) prior to submitting images to Coordinating Center.

Upon receipt, the Coordinating Center will verify that this information has been completely redacted, and, if necessary, will redact any remaining identifying information.

7.6.2 Ongoing Tumor Assessments

Patients who discontinue study treatment early during the initial treatment stage for reasons other than disease progression (e.g., toxicity) should continue to undergo scheduled tumor assessments (every 8 +/-1 weeks for the first 12 months following cycle 1, Day 1 and every 12 weeks thereafter) until the patient dies, experiences disease progression, withdraws consent, or until the study closes, whichever occurs first. Patients who start a new anti-cancer therapy in the absence of disease progression should continue to be followed for progression according to the protocol schedule of response assessments unless consent is withdrawn or the patient experiences disease progression or death or until study termination, whichever occurs first.

7.7 Laboratory

7.7.1 Screening

- Hematology (CBC, hemoglobin, hematocrit, WBC count with differential [neutrophils, eosinophils, lymphocytes], and platelet count)
- Serum chemistry (glucose, BUN or urea, creatinine, sodium, potassium, calcium, total bilirubin, direct bilirubin, ALT, AST, ALP, Lactate Dehydrogenase [LDH], lipase and amylase,)
- CEA
- Serology (HIV, HBsAg, antibodies against HBsAg, total HBcAg antibody [anti-HBcAb], HCV antibody [anti-HCV]). HBV DNA should be obtained prior to randomization if patient has a negative serology for HBsAg and a positive serology for anti-HBcAb.
  HCV RNA should be obtained prior to randomization if patient tests positive for anti-HCV
- Thyroid function testing (TSH, free T3, free T4)
- Serum pregnancy test within 14 days before Cycle 1 Day 1 for women of childbearing potential, including women who have had a tubal ligation.
  A woman is not considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (defined as 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone permanent surgical sterilization (removal of bilateral ovaries and/or uterus.
- Coagulation (INR and aPTT)

7.7.2 Study treatment period

- Hematology (CBC, hemoglobin, hematocrit, WBC count with differential [neutrophils, eosinophils, lymphocytes], and platelet count) will be performed before treatment administration
- Serum chemistry (glucose, BUN or urea, creatinine, sodium, potassium, calcium, total bilirubin, direct bilirubin, ALT, AST, ALP, LDH, lipase, amylase, and LDH) will be collected before treatment administration
- Thyroid function testing includes TSH, free T3, free T4 and it will be collected at Day 1 of every cycle during the second treatment phase.
- CEA will be done every 8 weeks (± 1 week) of the second treatment phase until disease progression leading to treatment discontinuation. After 12 months of treatment, it will be performed every 12 weeks.

7.8 Electrocardiograms

A twelve-lead ECG is required at screening and as clinically indicated. ECGs should be obtained on the same machine whenever possible. Lead placement should be as consistent as possible.

Paper or electronic copies of ECG tracings will be kept as part of the patient's permanent study file at the site. Any morphologic waveform changes or other ECG abnormalities must be documented on the eCRF.

7.9 Patient reported outcomes (PRO)

PRO data will be elicited from the patients in this study to more fully characterize the quality of life of patients. The following PRO instruments will be used, as shown in Appendix VI:

- EORTC QLQ-C30 (see Appendix VI)
- EORTC QLQ-CR29 (see Appendix VI)
- EuroQol EQ-5D-5L (see Appendix VI)

The PRO instruments will be distributed by the study staff and completed in their entirety by the patient. To ensure instrument validity and that data standards meet health authority requirements, PRO questionnaires should be self-administered at the investigational site prior to the administration of study treatment. PRO questionnaires should be performed every 8 weeks ± 7 days during treatment until PD leading to treatment discontinuation, regardless of whether study treatment has been administered or held, and also if the patient is off treatment due to reason other than PD (i.e. refusal or unacceptable toxicity). After 12 months of treatment, PRO questionnaires will be performed every 12 weeks.

In the event a study visit is conducted by telephone, the PRO data for that visit will be collected via telephone interview and recorded by the investigative staff. To maintain validity and minimize patient burden, the PRO instruments administered via telephone interview will consist of a reduced version of the EORTC QLQ (Items 1−7, 10, 12, 13, 16, 17, 18, 29, and 30 from the C30 and the two additional items from the item bank) and the telephone interview version of the EQ-5D-5L.

7.10 Tumor Tissue Samples

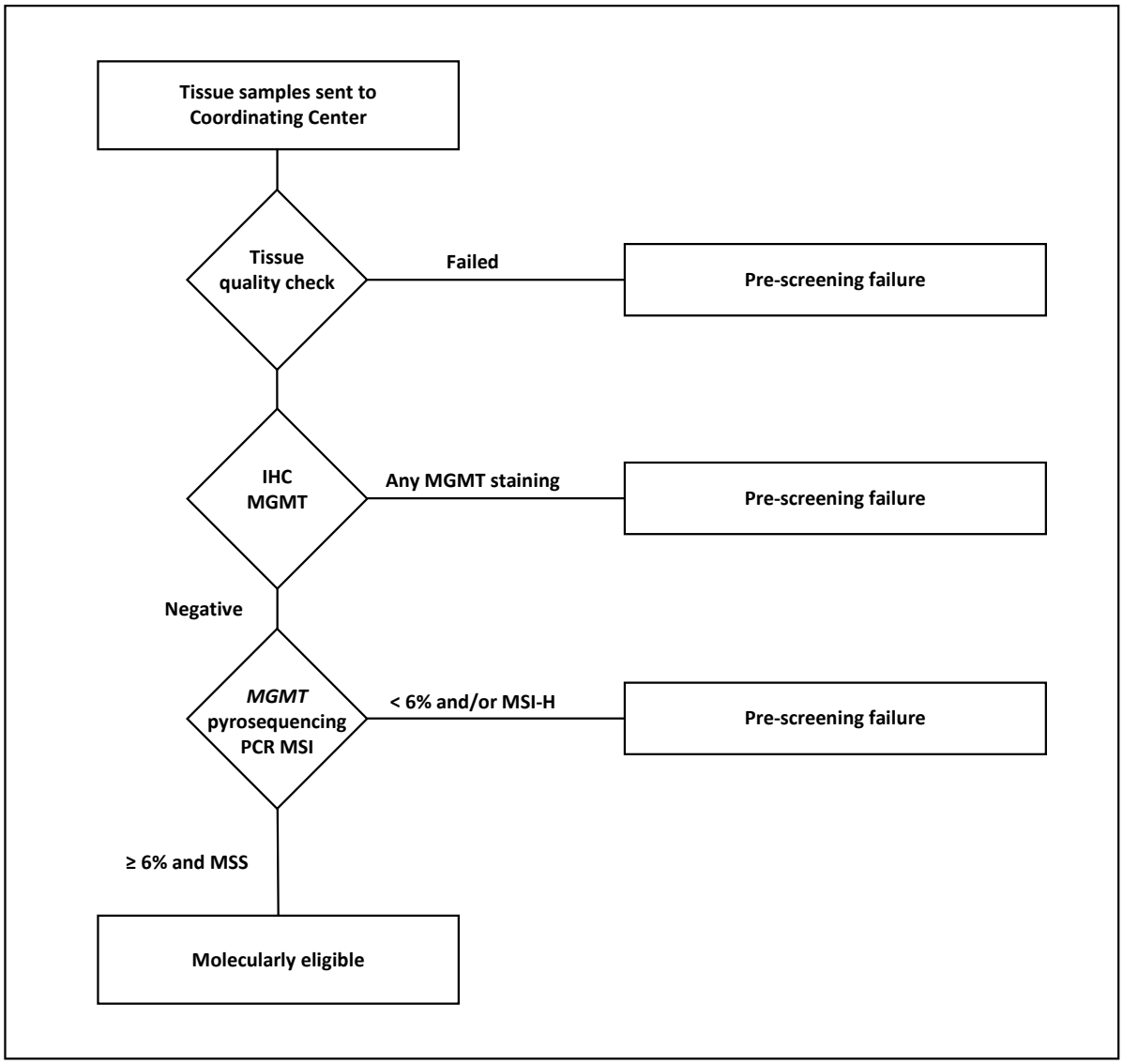
A central laboratory will coordinate the sample collection of tissue samples for screening and research related testing. The assessments will be performed by the Sponsor at the Department of Pathology of Fondazione IRCCS Istituto Nazionale dei Tumori. The remaining tumor tissue block will be returned to the site upon request. Instruction manuals and supply kits will be provided for all central laboratory assessments.See the laboratory manual for additional details on tissue sample handling.

Note: A separate tissue screening informed consent form may be used to obtain consent to send the sample to the central laboratory. Subjects may continue on prior therapy while tissue testing takes place.

7.10.1 Archival Tumor Sample for Screening

Representative tumor specimens in paraffin blocks (preferred) or at least 25 unstained sections on slides (see laboratory manual for details of tissue collection and handling) of tumor must be submitted for central confirmation of mismatch repair proficiency (microsatellite stable [MSS]) by multiplex polymerase chain reaction (PCR), MGMT promoter methylation by pyrosequencing and MGMT low expression by IHC. Results will be provided within a maximum of 14 days to the Study Centers. Archival tumor tissue for molecular screening will be obtained up to 28 days before initiation of study treatment. Molecular screening will be performed as per Figure 2.

Figure 2 Molecular screening



Archival tumor tissue should be collected as follows:

- A representative formalin−fixed, paraffin-embedded (FFPE) tumor specimen collected at first diagnosis and/or subsequent tumor recurrence(s) consistent with the patient's diagnosis is required for participation in this study (FFPE block [preferred], or a minimum of 25 unstained sections on slides of tumor will be required. This specimen must be accompanied by the associated pathology report.
- The available tumor sample must be adequate to determine extended MGMT status and MSS status.
- The tumor sample and associated pathology report must be confirmed to be available prior to any study−specific screening procedures. Fine-needle aspiration, brushing, cell pellet from pleural effusion, and lavage samples are not acceptable. For core needle biopsy specimens, at least three cores should be submitted for evaluation.
- For samples that do not meet the minimum requirements for size/slide number, contact the Sponsor via site contact with tissue size and tumor content/number of slides to determine eligibility.

– Alternatively, or if the archival tumor sample does not meet minimum requirements, the patient may be offered the option of undergoing a pretreatment procedure (excisional or core tumor biopsy) to obtain an adequate tumor sample, provided that his or her disease is easily accessible and tumor biopsies can be performed with minimal risk and discomfort.

If archival tissue is unavailable, a pretreatment tumor biopsy is required.

Cytological or fine-needle aspiration samples are not acceptable. Acceptable samples include:

– Core needle biopsies for deep tumor tissue; at least three cores, embedded into a single paraffin block, should be submitted for evaluation.
– Excisional or incisional tumor biopsy
– Tumor tissue resection

7.11 Treatment Discontinuation Visit

Patients who discontinue early from treatment for progression will be asked to return to the clinic not more than 30 days after the last treatment for a treatment discontinuation visit. The visit at which a response assessment shows progressive disease that results in patient discontinuation may be used as the treatment discontinuation visit.

See the study flowcharts provided in Appendix I for assessments to be performed at the treatment discontinuation visit.

7.12 Adverse Events

The definitions of an AE or serious adverse event (SAE) can be found in Section 8

All adverse events will be recorded until 30 days after the last dose of study treatment, and SAEs and adverse events of special interest (AESIs) will be recorded until 90 days after last dose of study treatment.

Ongoing adverse events thought to be related to study treatment will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, new anti-cancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or it has been determined that study treatment or participation is not the cause of the adverse event.

7.12.1 Survival and Subsequent Anti-Cancer Therapy Follow-Up

Following early discontinuation or completion of the treatment period, all patients will be followed for survival and subsequent anti-cancer therapy beginning 3 months after the end of treatment visit. Survival andsubsequent anti-cancer therapy follow-up information will be collected via telephone calls, patient medical records, and/or clinic visits approximately every 3 months until death, loss to follow-up, withdrawal of consent, or study termination by the Sponsor.

7.13 Exploratory studies

The exploratory objectives for this study are as follows:

Optional tumor re-biopsy prior to study entry and at disease progression will be offered, if feasible, in consenting patients. Longitudinal plasma samples (liquid biopsy and PBMCs) will be collected at baseline and every 4-week cycle until best response according to ir-RECIST; after that, they will be collected every 8 +/-1 weeks for the first 12 months and every 12 weeks thereafter and at disease progression leadingto treatment discontinuation. Quantification of the percentage of MGMT methylation (by digital PCR based methods) and MGMT IHC will be performed in archival tumor tissue and tumor re-biopsies. Moreover, digital PCR for MGMT methylation status will be performed in cell-free circulating DNA (cfDNA).

Mutational load will be assessed in archival tumor tissues, tumor biopsies and cfDNA before and during treatment by next-generation sequencing of exome or large gene panels (including DNA mismatch repair genes and microsatellite loci). Custom algorithms will be applied to count the number of G>A or C>T transitions, which are known genomic marks of TMZ exposure. We will then assess and rank the immunogenic value of all nucleotide variants induced by TMZ treatment using available bioinformatic tools (e.g. NetChop Cterm and NetMHC algorithms) that will inform about whether and to what extent chemotherapy-induced molecular alterations could result in the generation of neo-epitopes. Immune-related tissue and circulating biomarkers will also be studied. Analysis of PD-L1 will be performed on archival or new pre-treatment biopsies by IHC using the Dako anti PD-L1 antibody clone 28-8. Positivity is defined as membranous PD-L1 expression in more than 5% of neoplastic cells. Scoring of PD-L1 staining in tumor- infiltrating lymphocytes (TILs) will also be performed. PD1 and other lymphocyte activation markers will bemeasured in peripheral blood lymphocyte (PBL) post-treatment and correlated with mutational load. Forthese analyses, PBL will be isolated at baseline and every four weeks in concomitance to cfDNA extraction and stored frozen until analysis. LymphoTrack® Dx next-generation sequencing (NGS) assays will be usedto detect T-cell receptor gamma (TRG) rearrangements in PBL taken post-treatment.

7.13.1 Confidentiality

Data generated from the exploratory studies must be available for inspection upon request by representatives of national and local health authorities.

Patient medical information associated with these specimens is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

7.13.2 Consent to Participate in the Translational Study on optional biopsies

The Informed Consent Form will contain a separate section that addresses participation in the Translational study on optional tumor biopsies obtained prior to study entry (-28 to -1) and/or prior to C3D1 of the second treatment phase and/or at the time of disease progression leading to treatment discontinuation. The investigator or authorized designee will explain to each patient the objectives, methods, and potential hazards of participation in the Translational study. Patients will be told that they are free to refuse to participate and may withdraw their specimens at any time and for any reason during the storage period. A separate, specific signature will be required to document a patient's agreement to provide optional specimens. Patients who decline to participate will not provide a separate signature.

The investigator should document whether the patient has given consent to participate by completing the optional Translational study Informed Consent eCRF.

7.13.3 Withdrawal from the exploratory studies

Patients who give consent to provide specimens have the right to withdraw their specimens from the exploratory studies at any time for any reason. If a patient wishes to withdraw consent to the testing of his or her specimens, the investigator must inform the Sponsor in writing of the patient's wishes. The patient will be provided with instructions on how to withdraw consent after the trial is closed. A patient's withdrawal from the main Study does not, by itself, constitute withdrawal of specimens from the exploratory studies. Likewise, a patient's withdrawal from the exploratory studies does not constitute withdrawal from the main study.

7.14 Safety Assessments

7.14.1 Safety Plan

Measures will be taken to ensure the safety of patients participating in this trial, including the use of stringent inclusion and exclusion criteria (see Sections 4.1 and 4.2) and close monitoring of clinical conditions and laboratory tests (as indicated in Appendix I).

Administration of NIVO and IPI will be performed in a setting with emergency medical facilities and staff who are trained to monitor for and respond to medical emergencies. All adverse events and serious adverse events will be recorded during the trial and for up to 30 days after the last dose of study treatment or until the initiation of another anti-cancer therapy, whichever occurs first. Investigators are instructed to report all events (adverse events, pregnancy-related adverse events) considered related to study treatment regardless of time after study. A summary of the safety data available on TMZ, NIVO and IPI can be found in Section 1.4.3.1 The potential safety issues anticipated in this trial, as well as measures intended to avoid or minimize such toxicities, are outlined in the following sections.

7.14.1.1 General Plan to Manage Safety Concerns

7.14.1.1.1 Eligibility Criteria

Eligibility criteria were selected to guard the safety of patients in this trial. Results from the nonclinical toxicology studies and clinical data with TMZ, NIVO and IPI, as well as the nonclinical/clinical data from other checkpoint inhibitors, were taken into account. Specifically, patients at risk for study-emergent autoimmune conditions or with a prior diagnosis of autoimmune disease, patients with evidence of acute infections, and patients who have received a live-attenuated viral vaccine within 4 weeks before Day 1 are excluded from the study (see Section 4.2).

7.14.1.1.2 Monitoring

Safety will be evaluated in this study through the monitoring of all serious and non-serious adverse events, defined and graded according to NCI CTCAE v4.0. Patients will be assessed for safety (including laboratory values) according to the schedule in Appendix I. Laboratory values must be reviewed prior to each infusion.

General safety assessments will include serial interval histories, physical examinations, and specific laboratory studies, including serum chemistries and blood counts (see Appendix I for the list and timing of study assessments).

During the study, patients will be closely monitored for the development of any signs or symptoms of autoimmune conditions and infection.

All serious adverse events and protocol-defined events of special interest (see Sections 8.1 and 8.2) will be reported in an expedited fashion (see Section 8.3). In addition, the Sponsor will review and evaluateobserved adverse events on a regular basis.

Patients will be followed for safety for 30 days following their last dose of study treatment or, in case of SAEs/AESIs, until 90 days after last dose of study treatment.

Patients who have an ongoing study treatment–related adverse event upon study completion or at discontinuation from the study will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, new anti-cancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or it has been determined that study treatment or participation is not the cause of the adverse event.

7.14.2 Safety Parameters and Definitions

Safety assessments will consist of monitoring and recording adverse events, including serious adverse events and non-serious adverse events of special interest, measurement of protocol-specified safety laboratory assessments, measurement of protocol-specified vital signs, and other protocol-specified tests that are deemed critical to the safety evaluation of the study.

Certain types of events require immediate reporting to the Sponsor, as outlined in Section 8.3.

8 ADVERSE EVENTS

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

- Related: There is a reasonable causal relationship between study drug administration and the AE.
- Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

8.1 Serious Adverse Events

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy, overdose, potential drug-induced liver injury (DILI), and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (e.g., death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

NOTE:

The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department < 24 hours that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)
- SAEs occurring during lead-in phase with TMZ alone that are due to disease progression (non-drug related) should not be reported.

8.2 Adverse Events of Special Interest

Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

Adverse events of special interest are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 8.3 for reporting instructions). Adverse events of special interest for this study include the following:

- Cases of potential drug-induced liver injury that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice (see Section 8.9)
- Suspected transmission of an infectious agent by the study treatment, as defined below
  Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of study treatment is suspected.
- Pneumonitis
- Colitis
- Endocrinopathies: diabetes mellitus, pancreatitis, adrenal insufficiency, hyperthyroidism, and hypophysitis
- Hepatitis, including AST or ALT>10 x ULN
- Systemic lupus erythematosus
- Neurological disorders: Guillain-Barré syndrome, myasthenic syndrome or myasthenia gravis, and meningoencephalitis
- Events suggestive of hypersensitivity, infusion-related reactions, cytokine release syndrome, influenza-like illness, systemic inflammatory response syndrome, and systemic immune activation
- Nephritis

- Ocular toxicities (e.g., uveitis, retinitis)
- Myositis
- Myopathies, including rhabdomyolysis
- Grade ≥ 2 cardiac disorders (e.g., atrial fibrillation, myocarditis, pericarditis)
- Vasculitis

8.3 Serious Adverse Event Collection and Reporting

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- Serious adverse events
- adverse events of special interest
- Pregnancies

However, SAEs occurring during lead-in phase with TMZ alone that are due to disease progression (non- drug related) should not be reported.

The investigator must report new significant follow-up information for these events to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results
- Change in causality on the basis of new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event

Investigators must also comply with local requirements for reporting serious adverse events to the local health authority and IRB/EC.

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 90 days of discontinuation of dosing must be reported to the Sponsor and BMS Worldwide Safety, whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (e.g., a follow-up skin biopsy).
- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;

If the investigator believes that an SAE is not related to study drug but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

- The Sponsor will reconcile the clinical database AE cases (case level only) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms.com).

- The Sponsor will request from BMS GPV&E, aepbusinessprocess@bms.com the SAE reconciliation report and include the BMS protocol number every 3 months and prior to data base lock or final data summary
- GPV&E will send the investigator the report to verify and confirm all SAEs have been transmitted to the Sponsor and BMS GPV&E.
- The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to the Sponsor and to BMS (Worldwide.Safety@bms.com).

- In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).
- In accordance with local regulations, BMS will notify sponsor investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (i.e., not previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Sponsor investigator notification of these events will be in the form of either a SUSAR Report or a Semi-Annual SUSAR Report.
  - ✓ Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lackof efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (e.g., animal) study, important safety recommendations from a study data monitoring committee, or sponsor or BMS decision to end or temporarily halt a clinical study for safety reasons.
  - ✓ Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

SAEs, whether related or not related to study drug, and pregnancies must be reported to the Sponsor and to BMS within 24 hours \ 1 Business Day of becoming aware of the event. SAEs must be recorded on CIOMS form provided in Appendix VII.

Pregnancies must be reported and submitted to the Sponsor and BMS. BMS will perform due diligence follow-up using the BMS Pregnancy Form which the investigator must complete.

SAE Email Address: the SAE should be sent to both of the following email addresses:

farmacovigilanza.studispontanei@istitutotumori.mi.it

AND

Worldwide.Safety@BMS.com

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours \ 1 Business Day to the Sponsor and BMS using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

8.3.1 Emergency Medical Contacts

Filippo Pietrantonio:
Email: filippo.pietrantonio@istitutotumori.mi.it
Tel 0223903807; mobile 3466217748; fax 0223902149

Federica Palermo:
Email: federica.palermo@istitutotumori.mi.it
Tel 0223903835/2961

8.3.2 Reporting Requirements for Serious Adverse Events and Adverse Events of Special Interest

For reports of serious adverse events and adverse events of special interest, investigators should record all case details that can be gathered immediately (i.e., within 24 hours) on the Adverse Event eCRF and submit the report via the EDC system. A report will be generated and sent to the Sponsor's Safety Risk Management department by the EDC system.

In the event that the EDC system is unavailable, a paper Serious Adverse Event/Non-Serious Adverse Event of Special Interest CRF and Fax Coversheet should be completed and sent to both BMS and Sponsor immediately (i.e. within 24 hours) to this mailbox and fax (see Appendix VII).

Fondazione IRCCS Istituto Nazionale dei Tumori	farmacovigilanza.studispontanei@istitutotumori.mi.it
Pharmacist: Dr.ssa Gabriella Saibene Fondazione IRCCS Istituto Nazionale dei Tumori, Via G.Venezian n.1, 20133 Milano	Tel: 02.23903189 Fax: 02.23903189

BMS	worldwidesafety@bms.com

BMS GPV&E can send queries for follow-up information at: farmacovigilanza.studispontanei@istitutotumori.mi.it.

8.4 Non-serious Adverse Events

A ***non-serious adverse event*** is an AE not classified as serious.

8.5 Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information should begin at initiation of study drug. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 30 days following the last dose of study treatment.

Patients who have an ongoing study treatment-related adverse event upon study completion or at discontinuation from the study will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, new anti-cancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or it has been determined that study treatment or participation is not the cause of the adverse event.

Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [e.g., IND US trial] as part of an annual reporting requirement.

Non-serious AE information should also be collected from the start of the lead-in treatment period.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

8.6 Laboratory Test Result Abnormalities

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported to the Sponsor as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (e.g., anemia versus low hemoglobin value).

8.7 Pregnancy

A pregnancy report will automatically be generated and sent to Safety Risk Management. Pregnancy should not be recorded on the Adverse Event eCRF.

Pregnancies must be reported and submitted to BMS. BMS will perform due diligence follow-up using the BMS Pregnancy Form which the investigator must complete.

The investigator should discontinue study drug and counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any serious adverse events associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the Adverse Event eCRF.

If, following initiation of the investigational product, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner.

The investigator must immediately notify the Sponsor in accordance with SAE reporting procedures.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported to the Sponsor via EDC. Any pregnancy that occurs in a female partner of a male study participant should be reported to the Sponsor. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

8.8 Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as an SAE.

8.9 Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs (see Section 8.3 and Appendix VII for reporting details).

For participants with normal ALT, AST, and Total bilirubin at baseline, potential DILI is defined as:

1) AT (ALT or AST) elevation > 3× upper limit of normal (ULN) AND
2) Total bilirubin > 2× ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase), AND
3) No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

For participants with elevated AST or ALT or Total bilirubin at baseline, potential DILI is defined as:

1) AT (ALT or AST) elevation > 2× baseline AND 3× ULN; OR AT elevation 8 times ULN
   AND
2) Total bilirubin > 2× baseline AND >2× ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),
   AND
3) No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

8.10 Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

9 DATA MONITORING COMMITTEE AND OTHER EXTERNAL COMMITTEES

9.1 Data Monitoring Committee

A Data Monitoring Committee (DMC) will be used to evaluate safety during the study. Safety data will be reviewed by the DMC on a periodic basis, approximately every 3 months from the date of first-patient-in. In addition, the DMC will review safety data 28 days after the inclusion of the 6th patient. Safety data, including demographics, adverse events, serious adverse events, and relevant laboratory data, will be reviewed.

The DMC will provide a recommendation as to whether the study may continue, whether amendment(s) to the protocol should be implemented, or whether the study should be stopped. The final decision will rest withthe Sponsor.

9.2 Independent Central Review of Imaging

CD-ROM copies of the CT scans performed at baseline and during treatment until disease progression according to RECIST v1.1 and ir-RECIST criteria will be collected at the Coordinating Center (U.O. Oncologia Medica 1 Fondazione IRCCS Istituto Nazionale dei Tumori) for central review.

Site should follow their local privacy practices to de-identify all subject identifying information (name, medical record number, act.) prior to submitting images to the Coordinating Center.

Independent Central Review of imaging will be performed to assess response rate, DoR and PFS according to RECIST vers 1.1 and ir-RECIST, and to evaluate the safety of the combination.

10 STATISTICAL CONSIDERATIONS

10.1 Sample size determination

This is an open-label, multicenter, single-arm Phase II trial designed to estimate the activity of TMZ in association with NIVO and IPI in MGMT silenced, MSS, advanced CRC patients who have progressed after irinotecan, oxaliplatin, fluoropyrimidines and anti-EGFR mAbs (in *RAS/BRAF* wild type tumors).

According to our previously published results, in MGMT methylated mCRC with initial clinical benefit from TMZ (CR+PR+SD at 8 weeks) the individual PFS is almost always shorter than 6 months, and therefore the median PFS falls between 16- and 24-week reassessments. Therefore, after the first 8-week CT scan reassessment, by adding NIVO and IPI to TMZ, we plan to increase the 8-month PFS rate estimated by the Kaplan-Meier Method from 5% to 20%, with an alfa-error of 0.05 and a beta-error of 0.20.

According to the Fleming single-stage design and selecting the design parameters p0 (8-months PFS rate in the null hypothesis) = 0.05*, and p1 (8-months PFS rate in the alternative hypothesis) = 0.20, respectively, a total of 27 patients will be required. Null hypothesis will be rejected if at least 4 patients will be progression-free at the 8-months reassessment.

*A null hypothesis of 0.05 is based on the consideration that single agent TMZ does not lead to individual PFS times beyond 6 months in the refractory setting.

**The alternative hypothesis of 0.20 is considered as a potential target of interest for further studies in this setting with the experimental treatment.

10.2 Efficacy Analyses

10.2.1 Primary Efficacy Endpoint

The primary efficacy endpoint of this study is the 8-month PFS rate, defined as the proportion of patients alive and progression-free at 8 months from enrollment in the first treatment phase.

The primary endpoint will be 8-month (8-m) PFS in the 27 patients starting the second treatment phase. PFS status and date were assessed by the Investigators in each centre; PFS was defined as the interval from the date of enrollment in the first treatment phase to the date of first objective documentation of PD or deathfrom any cause, whichever occurred first; time was censored at the date of last follow up for patients alive and without PD. PFS will be assessed with the Kaplan-Meier method and 10-month estimates will be calculated and median (95% CI) time will be calculated.

10.2.2 Secondary Efficacy Endpoints

Secondary Efficacy Endpoints include:

- Investigator-assessed ORR per RECIST v1.1
- Investigator-assessed ORR per ir-RECIST (immune-related criteria)
- Investigator-assessed DOR per RECIST v1.1 and ir-RECIST
- Investigator-assessed PFS per RECIST v1.1 and ir-RECIST
- PRO
- Safety

OS was defined as the interval from the date of enrollment in the first treatment phase to the date of death from any cause or censored to the last follow up for patients alive. OS will be assessed with the Kaplan- Meier method and median (95% CI) time will be calculated. ORR was defined as the proportion of patients who achieved an objective response (CR or PR). DoR was defined as the time from the date of first documented response to the date of PD or death from any cause, whichever occurred first; time was censoredat the date of last follow up for patients alive and without PD. DOR will be assessed with the Kaplan-Meier method and median (95% CI) time will be calculated.

10.3 Overall response rate and Duration of Response as Determined by Investigator per RECIST v1.1 and Ir-RECIST

For the first endpoint (ORR), objective response is defined as a confirmed CR or PR as determined by the investigators per RECIST v1.1 (see Appendix III). Patients not meeting these criteria, including patients without at least one post-baseline response assessment, will be considered non-responders. For the second endpoint (ORRmod), ir-RECIST responses, which incorporate measurement of new lesions and confirmation by repeat assessment performed ≥ 28 days after the first criteria for response are first met, are described in detail in Appendix II. For the analysis according to ir-RECIST, patients not meeting these criteria, including patients without at least one post-baseline response assessment, will be considered non-responders.

DOR will be analyzed for the subset of *objective response-evaluable* patients who achieved an objective response as assessed by the investigator per RECIST v1.1 and Ir-RECIST. The DOR is defined as thetime from the initial occurrence of documented CR or PR (whichever occurs first) until documented disease progression as determined by the investigator or death due to any cause, whichever occurs first.

DOR will be estimated using the Kaplan-Meier method. The 95% CIs for median duration of objective response will be computed using the Brookmeyer and Crowley method. *Patients who have not progressed or who have died at time of analysis will be censored at the last tumor assessment date.*

10.4 Progression Free Survival as determined by Investigator per RECIST v1.1 and Ir-RECIST

PFS is defined as the time from the first dose of TMZ to time of disease progression per RECIST v1.1 and Ir-RECIST as determined by the investigator or death due to any cause on study, whichever occurs first. Patients who have not experienced disease progression and are alive at the time of analysis will be censored at the time of the last tumor assessment.

The Kaplan-Meier method will be used to estimate PFS. The 95% CIs for median PFS were computed using the Brookmeyer and Crowley method. *Patients with no post-baseline tumor assessment will be censored at the time of first dose plus 1 day.*

A patient is considered to have disease progression by ir-RECIST if either of the following conditionsis met:

a) Ir-RECIST criteria for progression were met at a tumor assessment and no subsequent tumor assessment was performed
b) Ir-RECIST criteria for progression were met at a tumor assessment and at the subsequenttumor assessment the criteria for confirmed progression by ir-RECIST were also met

For patients who meet criterion a), the date of progression is the date of the tumor assessment that met the criteria for ir-RECIST. For patients who meet criterion b), the date of progression is the date of the tumor assessment at which the ir-RECIST criteria for progression were first met. Patients who do not meet either of the above criteria are not considered to have had disease progression by ir-RECIST.

Patients who have not experienced disease progression and are alive at the time of analysis will be censored at the time of the last tumor assessment. The Kaplan-Meier method will be used to estimate PFS. The 95% CIs for median PFS will be computed using the Brookmeyer and Crowley method. Patients with no post- baseline tumor assessment will be censored at the time of first dose plus 1 day.

10.5 Overall Survival

OS is defined as the time from the first dose of TMZ to the time of death from any cause on study. Patients who are still alive at the time of analysis will be censored at the time of their last study assessment (for active patients) or at the last date known alive (for patients in follow-up). *The* Kaplan-Meier estimate of *the median* OS will be presented, along with the 95% CIs for median OS computed using Brookmeyer and Crowley formula. Kaplan-Meier methods will be used to estimate 1-year OS, along with the corresponding 95% CIs constructed using Greenwood's formula for the standard error.

10.6 PRO

The analysis of PRO endpoints (assessed using the EORTC QLQ-C30, the EORTC QLQ-CR29 and theEuroQol EQ-5D questionnaires) will be performed according to the EORTC Scoring and Reference Values Manual. All scores and subscales will be assessed through descriptive summary statistics.

10.7 Safety Analysis

Safety will be assessed through summaries of adverse events (including protocol-defined events of special interest), changes in laboratory test results, changes in vital signs, and exposure to TMZ, IPI and NIVO.

Verbatim descriptions of adverse events will be mapped to thesaurus terms using the MedDRA dictionary. Adverse event data will be listed by study site, patient number, and study day. Events occurring on the day or after administration of the first dose of treatment will be summarized by thesaurus term, appropriate thesaurus levels, and NCI CTCAE v4.0 grade. Serious adverse events, including deaths, will be listed

separately and will be summarized. For events of varying severity, the highest grade will be used in summaries.

Relevant laboratory tests and vital signs (heart rate, respiratory rate, blood oxygen saturation, blood pressures, and temperature) data will be displayed by time, with Grade 3 and 4 values identified, where appropriate. Additionally, all laboratory data will be summarized by NCI CTCAE v4.0 grade.

10.7.1 Data Monitoring Committee (DMC)

A Data Monitoring Committee (DMC) will be used to evaluate safety during the study. Safety data will be reviewed by the DMC on a periodic basis, approximately every 3 months from the date of first-patient-in. In addition, the DMC will review safety data 28 days after the inclusion of the 6th patient. Safety data, including demographics, adverse events, serious adverse events, and relevant laboratory data, will be reviewed.

The DMC will provide a recommendation as to whether the study may continue, whether amendment(s) to the protocol should be implemented, or whether the study should be stopped. The final decision will rest withthe Sponsor.

10.8 Exploratory Analyses

Exploratory biomarker analyses will be performed in an effort to understand the association of these markers with study drug sensitivity and/or resistance.

The exploratory outcome measures for this study are as follows:

Longitudinal plasma samples (liquid biopsy and PBMCs) will be collected at baseline and at every cycle until best response according to ir-RECIST, then every 8 +/-1 weeks for the first 12 months and every12 weeks thereafter and at disease progression leading to treatment discontinuation. Fresh-frozen and FFPE tumor tissue will be collected through optional tumor re-biopsy (if feasible and in consenting patients who sign a separate informed consent form) at baseline and/or prior to C3D1 of the second treatment phase and/orat disease progression leading to treatment discontinuation.

Quantification of the percentage of MGMT methylation (by digital PCR based methods, i.e. methyl-BEAMing) and MGMT IHC will be performed in archival tumor tissue and tumor re-biopsies.

Digital PCR for MGMT methylation status will be performed in cell-free circulating DNA (cfDNA) (cfDNA).

Mutational load will be assessed in archival tumor tissues, tumor biopsies and cfDNA by means of whole exome sequencing.

Immune-related tissue and circulating biomarkers will also be studied.

10.9 Handling of Missing Data

For objective response (per ir-RECIST or RECIST v1.1), patients without a post-baseline tumor assessment will be considered non-responders.

For DOR, data from patients who did not experience disease progression or died will be included in the analysis as censored observations on the last date that the patient was known to be progression free, defined as the date of the last tumor assessment. If no tumor assessments were performed after the date of the first occurrence of a complete or partial response, duration of objective response will be censored at the date ofthe first occurrence of a complete or partial response (whichever occurs first) plus 1 day.

For the analysis of PFS, data for patients without disease progression or death will be censored at the date of the last tumor assessment (or, if no tumor assessments were made after the baseline visit, at the date of first

treatment plus 1 day). Data for patients who were lost to follow-up will be censored at the last date of tumor assessment at which the patient was known to be progression free.

For the analysis of OS, patients who are still alive at the time of analysis will be censored at the time of their last study assessment (for active patients) or at the last date known alive (for patients in follow-up).

11 STUDY MANAGEMENT

11.1 Study Documentation

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol, protocol amendments, Informed Consent Forms, and documentation of IRB/EC and governmental approval.

11.2 Protocol Violations

The investigator should document and explain any violations from the approved protocol. The investigator should promptly report any violations that might impact patient safety and data integrity to the Sponsor and to the IRB/EC in accordance with established IRB/EC policies and procedures.

11.3 Site Inspections

Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, patients' medical records, and eCRFs. The investigator will permit health authorities, Sponsor monitors, representatives, and collaborators and the IRBs/ECs to inspect facilities and records relevant to this study.

11.4 Protocol Amendments

Any protocol amendments will be prepared by the Sponsor. Protocol amendments will be submitted to the IRB/EC and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the IRB/EC and regulatory authorities before implementation of any changes, except for changes necessary to eliminate an immediate hazard to patients or changes that involve logistical or administrative aspects only (e.g., change in Sponsor or contact information).

11.5 Compliance with the Protocol and Protocol Revisions

The investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion of an amendment from the IRB/IEC (and if applicable, also by local health authority) except where necessary to eliminate an immediate hazard(s) to study subjects. If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining relevant approval/favorable opinion(s), the deviation or change will be submitted, as soon as possible to:

- IRB/IEC
- Regulatory Authority(ies), if applicable by local regulations (per national requirements). Documentation of approval/favorable opinion signed by the chairperson or designee of the IRB(s)/IEC(s) and if applicable, also by the local health authority must be sent to the Sponsor.

If an amendment substantially alters the study design or increases the potential risk to the participant: (1) the consent form must be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion; (2) the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and (3) the new form must be used to obtain consent from new subjects prior to enrolment. If the revision is done via an administrative letter, investigators must inform their IRB(s)/IEC(s).

11.6 Monitoring

Sponsor or designee representatives will review data centrally to identify potential issues to determine a schedule of on-site visits for targeted review of study records. Representatives of the Sponsor must be allowed to visit all study site locations periodically to assess the data quality and study integrity. On site they will review study records and directly compare them with source documents, discuss the conduct of the studywith the investigator, and verify that the facilities remain acceptable. Certain CRF pages and/or electronic files may serve as the source documents. In addition, the study may be evaluated by Sponsor or designee internal auditors and government inspectors who must be allowed access to CRFs, source documents, other study files, and study facilities. The Sponsor audit reports will be kept confidential. The investigator must notify the Sponsor promptly of any inspections scheduled by regulatory authorities, and promptly forward copies of inspection reports to Sponsor or designee.

11.7 Source Documentation

The Investigator is responsible for ensuring that the source data are accurate, legible, contemporaneous, original and attributable, whether the data are hand-written on paper or entered electronically. If source data are created (first entered), modified, maintained, archived, retrieved, or transmitted electronically via computerized systems (and/or any other kind of electronic devices) as part of regulated clinical trial activities, such systems must be compliant with all applicable laws and regulations governing the use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records (EMRs/EHRs), adverse event tracking/reporting, protocol required assessments, and/or drug accountability records). When paper records from such systems are used in place of electronic format to perform regulated activities, such paper records should be certified copies. A certified copy consists of a copy of original information that has been verified, as indicated by a dated signature, as an exactcopy having all of the same attributes and information as the original.

11.8 Records

11.9 Records Retention

The investigator must retain all study records and source documents for the maximum period required by applicable regulations and guidelines, or institution procedures, or for the period specified by the Sponsor or designee, whichever is longer. The investigator must contact the Sponsor prior to destroying any records associated with the study. The Sponsor or designee will notify the investigator when the study records are no longer needed. If the investigator withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another investigator, study site, IRB). Notice of such transfer will be given in writing to the Sponsor or designee.

11.10 Study Drug Records

Records for study treatments (whether supplied by the Sponsor, its vendors, or the site) must substantiate study treatment integrity and traceability from receipt, preparation, administration, and through destructionor return. Records must be made available for review at the request of the Sponsor/designee or a Health Authority

11.11 Case Report Forms

An investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated in the investigation. Data that are derived from source documents and reported on the CRF must be consistent with the source documents or the discrepancies must be explained. Additional clinical information may be collected and

analyzed in an effort to enhance understanding of product safety. CRFs may be requested for AEs and/or laboratory abnormalities that are reported or identified during the course of the study. For sites using the Sponsor or designee electronic data capture tool, electronic CRFs will be prepared for all data collection fields except for fields specific to SAEs and pregnancy, which will be reported as described in Section 8.3. The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s). The investigator willmaintain a signature sheet to document signatures and initials of all persons authorized to make entries and/or corrections on CRFs. The completed CRF must be promptly reviewed, signed, and dated by the investigator or qualified physician, who is a subinvestigator and who is delegated this task on the Delegation of Authority Form. For electronic CRFs, review and approval/signature are completed electronically through the electronic data capture tool. The investigator must retain a copy of the CRFs including records of the changes and corrections. Each individual electronically signing electronic CRFs must meet Sponsor or designee training requirements and must only access the electronic data capture tool using the unique user account provided by Sponsor or designee. User accounts are not to be shared or reassigned to otherindividuals

11.12 Clinical Study Report and Publications

A Signatory Investigator must be selected to sign the clinical study report. For this protocol, the Signatory Investigator will be selected as appropriate based on the following criteria:

- Participant recruitment (e.g., among the top quartile of enrollers)
- Involvement in trial design
- Regional representation (e.g., among top quartile of enrollers from a specified region or country)
- Other criteria (as determined by the study team)

The data collected during this study are confidential and proprietary to Sponsor or designee. Any publications or abstracts arising from this study must adhere to the publication requirements set forth in the clinical trial agreement (CTA) governing [Study site or Investigator] participation in the study. These requirements include, but are not limited to, submitting proposed publications to Sponsor or designee at the earliest practicable time prior to submission or presentation and otherwise within the time period set forth in the CTA.

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APPENDIX I: SCHEDULE OF ASSESSMENTS

	1° Treatment Phase				2° Treatment Phase				Follow-up Phase	
Procedure	**SCR[a]**	**Cycle 1 (q28d)**	**Cycle 2 (q28d)**	**End 1° Tx phase**	**Cycle 1 (q28d)**	**Cycle 2 (q28d)**	**Cyclex* (q28d)**	**Cycley** (q28d)**	**Tx discontinuation[b]**	**Survival FU[c]**
Day	-28 to -1	1	1	+/- 7 days	1	1	1	1	< 30 d after last dose	q3 mos
Informed consent[d]	x									
Inclusion/ Exclusion criteria	x									
Archival tumor tissue[e]	x									
Demographics	x									
Medical and CRC history	x									
Concomitant medications	x	x	x		x	x	x	x	x	x
Vital signs[f,g]	x	x	x		x	x	x	x	x	
ECOG PS	x	x	x		x	x	x	x	x	x
Weight	x	x	x		x	x	x	x	x	
Height	x									
Physical examination	x	x	x		x	x	x	x	x	
Hematology[h]	x	x	x		x	x	x	x	x	
Coagulation [i]	x								x	
Chemistry[j]	x	x	x		x	x	x	x	x	
CEA[k]	x				CEA will be done every 8 weeks After 12 months, every 12 weeks until confirmed disease progression.					
ECG	x	As clinically indicated			As clinically indicated					
Serology[m]	x									
Thyroid function[n]	x				x	x	x	x	x	
Pregnancy test[o]	x									
Adverse events[p]	Assess continuously				Assess continuously					
Tumor Assessments[l]	x			x	Scans will be done every 8 weeks. After 12 months, every 12 weeks until confirmed disease progression.					
EORTC QLQC30 EQ-5D-5L[q] EORTC QLQ-CR29	x			x	PRO will be done every 8 weeks. After 12 months, every 12 weeks until confirmed disease progression.				x	
Survival and anti-cancer therapy follow-up										x[s]
Tumor biopsy [r]					Patients will undergo an optional tumor biopsy sample collection, if clinically feasible as assessed by investigators, prior to C3D1				x	
Biomarker Blood Samples[t]		x	x		Every 4 weeks before best response, then every 8 weeks				x	
Nivolumab Administration[u]					x	x	x	x		
Temozolomide Administration[v]		x	x		x	x	x	x		

Ipilimumab Administration[w]					x		x			

SCR= screening; CEA= carcinoembryonic antigen; CRC= colorectal cancer; d = day; ECOG PS=Eastern Cooperative Oncology Group Performance Status; EORTC QLQ-C30 =European Organization for Research and Treatment of Cancer Quality of Life-C30 – c29 questionnaire; EQ-5D-5L =EuroQoL 5 Dimensions; FU= follow-up; q3m = every 3 months; Tx= treatment

[*] To be repeated as Cycle 1, every 8 weeks
[**] To be repeated as Cycle 2, every 8 weeks

[a] Results of standard of care tests or examinations performed prior to obtaining informed consent and within 28 days prior to Day 1 may be used; such tests do not need to be repeated for screening.
[b] Patients who discontinue study drug will return to the clinic for a treatment discontinuation visit.
[c] Required follow-up information will be collected via telephone calls and/or clinic visits every 3 months until death, withdrawal of consent, the patient is lost to follow-up, or study termination, whichever occurs first.
[d] Informed consent must be documented before any study-specific screening procedure is performed and may be obtained up to 28 days before initiation of study treatment.
[e] Archival tumor tissue for centralized molecular screening will be obtained up to 28 days before initiation of study treatment. Note: A separate tissue screening informed consent form may be used to obtain consent to send the sample to the central laboratory. Subjects may continue on prior therapy while tissue testing takes place (see Section 7.10).
[f] Includes respiratory rate, heart rate, temperature, blood oxygen saturation and systolic and diastolic blood pressure while the patient is in a seated position. Any abnormality identified at baseline prior to the first treatment phase should be recorded on the General Medical History and Baseline Conditions eCRF. At subsequent visits record new or worsened clinically significant abnormalities on the Adverse Event eCRF.
[g] Vital signs at the first NIVO and IPI infusion will be collected within 60 min prior to the infusion and 30 (± 10) minutes after the infusion. During the infusion, the patient's vital signs will be determined only in presence of distress/infusion-related reactions. For subsequent infusions, vital signs will be collected within 60 minutes before infusion and at the end of the infusion.
[h] Hematology includes CBC, hemoglobin, hematocrit, WBC count with differential [neutrophils, eosinophils, lymphocytes], and platelet count. Hematology will be collected ≤ 48 hours prior to every treatment administration.
[i] Coagulation includes INR, aPTT. Coagulation will be collected at screening and before every optional biopsy.
[j] Serum chemistry includes: glucose, BUN or urea, creatinine, sodium, potassium, calcium, total bilirubin, direct bilirubin, ALT, AST, ALP, LDH, lipase, amylase, and LDH. Serum chemistry will be collected ≤ 48 hours prior to every treatment administration.
[k] CEA will be done at screening and every 8 weeks (± 1 week) until disease progression leading to treatment discontinuation. After 12 months of treatment, it will be performed every 12 weeks.
[l] Tumor assessments will be done at screening and every 8 weeks (± 1 week) until disease progression per RECIST v1.1, or ir-RECIST, or loss of clinical benefit (patients who continue treatment after disease progression according to ir-RECIST), consent withdrawal, or death, whichever occurs first. After 12 months, patients will undergo tumor assessments every 12 weeks until confirmed disease progression. Patients who discontinue treatment for reasons other than disease progression (e.g., toxicity) will continue scheduled tumor assessments until disease progression, withdrawal of consent, or death, whichever occurs first. A CT (with contrast if not contraindicated) or MRI scan of the brain must be doneat screening to exclude CNS metastases. An MRI scan of the brain is required to confirm or refute the diagnosis of CNS metastases at baseline in the event of an equivocal scan.
[m] All patients will be tested for HIV prior to the inclusion into the study and HIV-positive patients will be excluded from the clinical study. HBV serology will include HBsAg, antibodies against HBsAg, total HBcAg antibody (anti-HBcAb). HBV DNA should be obtained prior to the treatment phase if patient has a negative serology for HBsAg and a positive serology for anti-HBcAb. HCV serology will include HCV

antibody (anti-HCV). HCV RNA should be obtained prior to the treatment phase if patient tests positive for anti-HCV.

[n] Thyroid function testing includes TSH, free T3, free T4 and will be collected at screening and at every cycle of the second treatment phase.

[o] Serum pregnancy test within 14 days before Cycle 1 Day 1 of the first treatment phase.

[p] All Serious Adverse Events will be reported within 24 hours. All adverse events will be recorded until 30 days after the last dose of study treatment, and SAEs and AESIs will be recorded until 90 days after last dose of study treatment or initiation of new anti-cancer therapy, whichever occurs first. All other adverse events, regardless of relationship to study drug, will be reported until 30 days after the last dose of study drug or initiation of new anti-cancer therapy, whichever occurs first. After this period the investigator should report any serious adverse events or adverse events of special interest that are believed to be related to prior study drug.

[q] PRO instruments EORTC QLQ-C30 and EQ-5D-5L will be completed at screening and every 8 (± 1) weeks and at disease progression leading to treatment discontinuation. After 12 months of treatment, it will be performed every 12 weeks. In the event a study visit is conducted by telephone, the PRO data for that visit will be collected via telephone interview and recorded by the investigative staff. To maintain validity and minimize patient burden, the PRO instruments administered via telephone interview will consist of a reduced version of the EORTC QLQ (Items 1−7, 10, 12, 13, 16, 17, 18, 29, and 30 from the C30 and the two additional items from the item bank) and the telephone interview version of the EQ-5D-5L.

[r] Patients will undergo an optional tumor biopsy sample collection, if clinically feasible as assessed by investigators, at C3D1 of the second treatment phase and/or at the evidence of radiographic disease progression causing treatment discontinuation.

[s] Patients who discontinue treatment for reasons other than disease progression (e.g., toxicity) will continue scheduled tumor assessments until disease progression, withdrawal of consent, study termination, or death, whichever occurs first.

[t] Biomarker blood samples will be collected at baseline and before every cycle of Temozolomide administration until best response according to ir-RECIST; after that, they will be collected every 8 +/-1 weeks for the first 12 months and every 12 weeks thereafter and at disease progression leading to treatment discontinuation.

[u] Nivolumab dose is administered Q4W, within 3 days before or after the schedule date if necessary. There should be no less than 25 days between Nivolumab doses. The initial dose will be delivered over 60 (± 10) minutes. If the first infusion is well tolerated all subsequent infusions will be delivered over 30 (± 10) minutes until loss of clinical benefit.

[v] Temozolomide is administered daily on days 1-5 of every cycle, Q4W, within 3 days before or after the scheduled date if necessary.

[w] Ipilimumab dose is administered Q8W, within 3 days before or after the scheduled date if necessary. Ipilimumab infusions will be delivered over 30 (± 10) minutes.

APPENDIX II: Modified Response Evaluation Criteria in Solid Tumors

Conventional response criteria may not be adequate to characterize the anti-tumor activity of immunotherapeutic agents like NIVO or IPI, which can produce delayed responses that may be preceded by initial apparent radiological progression, including the appearance of new lesions. Therefore, modified response criteria have been developed that account for the possible appearance of new lesions and allow radiological progression to be confirmed at a subsequent assessment.

Ir-RECIST is derived from RECIST v1.1 conventions[1] and immune-related response criteria[2] (irRC). *When not otherwise specified, RECIST v1.1 conventions will apply.*

Table 10. Ir-RECIST and RECIST v1.1: Summary of Changes

	RECIST v1.1	Ir-RECIST
New lesions after baseline	Define progression	New measurable lesions are added into the total tumor burden and followed.
Non-target lesions	May contribute to the designation of overall progression	Contribute only in the assessment of a complete response
Radiographic progression	First instance of ≥ 20% increase in the sum of diameters or unequivocal progression in non-target disease	Determined only onthe basis of measurable disease; confirm*ation* by a *repeat* consecutive assessment ≥ 4 weeks from the date first documented

DEFINITIONS OF MEASURABLE/NON-MEASURABLE LESIONS

All measurable and non-measurable lesions should be assessed at Screening and at the protocol-specified tumor assessment timepoints. Additional assessments may be performed, as clinically indicated for suspicionof progression. The Investigator will evaluate response to treatment using ir-RECIST.

MEASURABLE LESIONS

Tumor Lesions. Tumor lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size as follows:

• 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (CT/MRI scan slice thickness/interval no greater than 5 mm)

• 10-mm caliper measurement by clinical examination (lesions that cannot be accurately measured with calipers should be recorded as non-measurable)

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in the short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and follow-up, only the short axis will be measured and followed.

NON-MEASURABLE LESIONS

Non-measurable tumor lesions encompass small lesions (longest diameter < 10 mm or pathological lymph nodes with short axis ≥ 10 but < 15 mm), as well as truly non-measurable lesions. Lesions considered truly non-measurable include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, peritoneal spread, and abdominal mass/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques.

SPECIAL CONSIDERATIONS REGARDING LESION MEASURABILITY

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment, as outlined below.

BONE LESIONS

Technetium-99m bone scans (T-99m), sodium fluoride−positron emission tomography (NaF-PET) scans, or plain films are not considered adequate imaging techniques for measuring bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

Lytic bone lesions or mixed lytic–blastic lesions, with identifiable soft tissue components, that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

Blastic bone lesions are non-measurable.

CYSTIC LESIONS

Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with Prior Local Treatment

Tumor lesions situated in a previously irradiated area or in an area subjected to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

TUMOR RESPONSE EVALUATION

DEFINITIONS OF TARGET/NON-TARGET LESIONS

Target Lesions

When more than one measurable lesion is present at baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline. This means that, for instances in which patients have only one or two organ sites involved, a maximum of two lesions (one site) and four lesions (two sites), respectively, will be recorded. Other lesions (albeit measurable) in those organs will be recorded as non-measurable lesions (even if the size is > 10 mm by CT scan).

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and be representative of all involved organs, but in addition, should lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement, in which circumstance, the next largest lesion that can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures that may be visible by imaging even if not involved by tumor. As noted above, pathological nodes that are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reportedas two dimensions in the plane in which the image is obtained (for CT, this is almost always the axial plane; for MRI, the plane of acquisition may be axial, sagittal, or coronal). The smaller of these measures is theshort axis. For example, an abdominal node that is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis of < 10 mm are considered non-pathological and should not be recorded or followed.

Lesions irradiated within 3 weeks prior to Cycle 1 Day 1 may not be counted as target lesions.

Non-Target Lesions

All other lesions (or sites of disease), including pathological lymph nodes, should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required.

It is possible to record multiple non-target lesions involving the same organ as a single item on the Case Report Form (CRF) (e.g., “multiple enlarged pelvic lymph nodes” or “multiple liver metastases”).

After baseline, changes in non-target lesions will contribute only in the assessment of complete response (i.e., a complete response is attained only with the complete disappearance of all tumor lesions, including non-target lesions) and will not be used to assess progressive disease.

New Lesions

During the study, all new lesions identified and recorded after baseline must be assessed at all tumor assessment timepoints. New lesions will also be evaluated for measurability with use of the same criteria applied to prospective target lesions at baseline per RECIST, (e.g., non−lymph node lesions must be ≥ 10mm; see note for new lymph node lesions below). Up to a maximum of five new lesions total (and a maximum of two lesions per organ), all with measurements at all timepoints, can be included in the tumor response evaluation. New lesion types that would not qualify as target lesions per RECIST cannot be included in the tumor response evaluation.

New lesions that are not measurable at first appearance but meet measurability criteria at a subsequent timepoint will be measured from that point on and contribute to the sum of longest diameters (SLD), if the maximum number of 5 measurable new lesions being followed has not been reached.

CALCULATION OF SUM OF THE DIAMETERS

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated as a measure of tumor burden.

The sum of the diameters is calculated at baseline and at each tumor assessment for the purpose ofclassification of tumor responses.

Sum of the Diameters at Baseline: The sum of the diameters for all target lesions identified at baseline prior to treatment on Day 1.

Sum of the Diameters at Tumor Assessment: For every on-study tumor assessment collected per protocol or as clinically indicated, the sum of the diameters at tumor assessment will be calculated using tumor imaging scans. All target lesions and all new measurable lesions that have emerged after baseline will contribute to the sum of the diameters at tumor assessment. Hence, each net percentage change in tumor

burden per assessment with use of ir-RECIST accounts for the size and growth kinetics of both old and new lesions as they appear.

Note: In the case of new lymph nodes, RECIST v1.1 criteria for measurability (equivalent to baseline target lesion selection) will be followed. That is, if at first appearance the short axis of a new lymph node lesion ≥ 15 mm, it will be considered a measurable new lesion and will be tracked and included in the SLD. Thereafter, the lymph node lesion will be measured at subsequent timepoints and measurements will be included in the SLD, even if the short axis diameter decreases to < 15 mm (or even < 10 mm). However, if it subsequently decreases to < 10 mm, and all other lesions are no longer detectable (or have also decreased toa short axis diameter of < 10 mm if lymph nodes), then a response assessment of CR may be assigned.

If at first appearance the short axis of a new lymph node is ≥ 10 mm and < 15 mm, the lymph node will not be considered measurable but will still be considered a new lesion. It will not be included in the SLD unless it subsequently becomes measurable (short axis diameter ≥ 15 mm).

The appearance of new lymph nodes with diameter < 10 mm should not be considered pathological and not considered a new lesion.

RESPONSE CRITERIA

Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Lymph nodes that shrink to < 10 mm short axis are considered normal.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of all target and all new measurable lesions, taking as reference the baseline sum of diameters, in the absence of CR.

Note: the appearance of new measurable lesions is factored into the overall tumor burden, but *does not automatically qualify as progressive disease* until the sum of the diameters increases by ≥ 20% when compared with the sum of the diameters at nadir.

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

Progressive Disease (PD): At least a 20% increase in the sum of diameters of all target and all new measurable lesions, taking as reference the smallest sum on study (*nadir SlD;* this includes the baseline sumif that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

Impact of New Lesions on Ir-RECIST

New lesions alone do not qualify as progressive disease. However, their contribution to total tumor burden is included in the sum of the diameters, which is used to determine the overall ir-RECIST tumor response.

EVALUATION OF BEST OVERALL RESPONSE USING IR-RECIST:

TIMEPOINT RESPONSE

It is assumed that at each protocol-specified timepoint, a response assessment occurs. Table 11 provides a summary of the overall response status calculation at each timepoint for patients who have measurable disease at baseline.

MISSING ASSESSMENTS AND *NOT* EVALUABLE DESIGNATION

When no imaging/measurement is done at all at a particular timepoint, the patient is not evaluable (NE) at that timepoint. If only a subset of lesion measurements are made at an assessment, usually the case is also

considered NE at that timepoint, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned timepoint response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and at follow-up only two lesions were assessed but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

Table 11 Ir-RECIST Timepoint Response Definitions

% Change in Sum of the Diameters (including measurable new lesions when present)	Target Lesion Definition	Non-Target Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	Overall Ir-RECIST Timepoint Response
−100% [a]	CR	CR	No	No	CR
−100% [a]	CR	Non-CR or not all evaluated	No	No	PR
≤−30%	PR	Any	Yes or no	Yes or no	PR
−30% to < + 20%	SD	Any	Yes or no	Yes or no	SD
Not all evaluated	Not evaluated	Any	Yes or no	Yes or no	NE
≥+ 20%	PD	Any	Yes or no	Yes or no	PD

CR = complete response; NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease.
[a] When lymph nodes are included as target lesions, the % change in the sum of the diameters may not be 100% even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm in order to meet the definition of CR.

BEST OVERALL RESPONSE: ALL TIMEPOINTS

The best overall response is determined once all the data for the patient are known.

The best overall response according to ir-RECIST is interpreted as below:

• **Complete Response (CR)**: Complete disappearance of all tumor lesions (target and non-target) and no new measurable or unmeasurable lesions, confirmed by a consecutive assessment ≥ 4 weeks from the date first documented. All lymph nodes short axes must be < 10 mm.

• **Partial Response (PR)**: Decrease in the sum of the diameters of all target and all new measurable lesions ≥ 30% relative to baseline, in the absence of CR, confirmed by a consecutive assessment ≥ 4 weeks from the date first documented.

• **Stable Disease (SD)**: Criteria for CR, PR, and PD are not met.

• **Progressive Disease (PD)**: Increase in the sum of the diameters of all target and all new measurable lesions ≥ 20% relative to the nadir, which may be confirmed by a consecutive assessment ≥ 4 weeks from the date first documented as follows:

This protocol allows patients to continue to receive study treatment even after confirmed radiographic PD per ir-RECIST, and patients may achieve a best overall response of PR or CR based on tumor regression achieved at any time prior to study treatment discontinuation.

APPENDIX III Response Evaluation Criteria in Solid Tumors (RECIST v 1.1)

Selected sections from the Response Evaluation Criteria in Solid Tumors (RECIST) (Eisenhauer EA et al), Version 1.11 are presented below, with slight modifications and the addition of explanatory text as neededfor clarity.

MEASURABILITY OF TUMOR AT BASELINE

DEFINITIONS

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows.

a. Measurable Tumor Lesions

Tumor Lesions. Tumor lesions must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

• 10 mm by computed tomography (CT) or magnetic resonance imaging (MRI) scan (CT/MRI scan slice thickness/interval no greater than 5 mm)

• 10-mm caliper measurement by clinical examination (lesions that cannot be accurately measured with calipers should be recorded as non-measurable)

• 20 mm by chest X-ray

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in the short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed. See also notes below on "Baseline Documentation of Target and Non-Target Lesions" for information on lymph node measurement.

b. Non-Measurable Tumor Lesions

Non-measurable tumor lesions encompass small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), as well as truly non-measurable lesions. Lesions considered truly non-measurable include leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, peritoneal spread, and abdominal masses/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques.

c. Special Considerations Regarding Lesion Measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment, as outlined below.

Bone lesions:

• Technetium-99m bone scans (T-99m), sodium fluoride−positron emission tomography (NaF-PET) bone scans, or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

• Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered measurable lesions if the soft tissue component meets the definition of measurability described above.

• Blastic bone lesions are non-measurable.

Cystic lesions:

• Lesions that meet the criteria for radiographically defined simple cysts should not be considered malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

• Cystic lesions thought to represent cystic metastases can be considered measurable lesions if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

• Tumor lesions situated in a previously irradiated area or in an area subjected to other loco-regional therapy are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

TARGET LESIONS: SPECIFICATIONS BY METHODS OF MEASUREMENTS

a. Measurement of Lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

b. Method of Assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during study. Imaging-based evaluation should always be the preferred option.

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

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

CT, MRI. CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan on the basis of the assumption that CT slice thickness is 5 mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable.

If prior to enrollment it is known that a patient is unable to undergo CT scans with intravenous (IV) contrast because of allergy or renal insufficiency, the decision as to whether a non-contrast CT or MRI (without IV contrast) will be used to evaluate the patient at baseline and during the study should be guided by the tumor type under investigation and the anatomic location of the disease. For patients who develop contraindications to contrast after baseline contrast CT is done, the decision as to whether non-contrast CT or MRI (enhanced or non-enhanced) will be performed should also be based on the tumor type and the anatomic location of the disease and should be optimized to allow for comparison with the prior studies if possible. Each case should be discussed with the radiologist to determine if substitution of these other approaches is possible and, if not, the patient should be considered not evaluable from that point forward. Care must be taken in measurement of target lesions on a different modality and interpretation of non-target disease or new lesions since the same lesion may appear to have a different size using a new modality.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement.

Endoscopy, Laparoscopy, Tumor Markers, Cytology, Histology. The utilization of these techniques for objective tumor evaluation cannot generally be advised.

TUMOR RESPONSE EVALUATION

ASSESSMENT OF OVERALL TUMOR BURDEN AND MEASURABLE DISEASE

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and to use this as a comparator for subsequent measurements. Measurable disease is defined by the presence of at least one measurable lesion, as detailed above.

BASELINE DOCUMENTATION OF TARGET AND NON-TARGET LESIONS

When more than one measurable lesion is present at baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified astarget lesions and will be recorded and measured at baseline. This means in instances where patients have only one or two organ sites involved, a maximum of two lesions (one site) and four lesions (two sites), respectively, will be recorded. Other lesions (albeit measurable) in those organs will be recorded as non- measurable lesions (even if the size is > 10 mm by CT scan).

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and be representative of all involved organs but, additionally, should lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement, in which circumstance the next largest lesion that can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures that may be visible by imaging even if not involved by tumor. As noted above, pathological nodes that are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reportedas two dimensions in the plane in which the image is obtained (for CT scan, this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal, or coronal). The smaller of these measures is the short axis. For example, an abdominal node that is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as thenode measurement. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed.

Lesions irradiated within 3 weeks prior to Cycle 1, Day 1 may not be counted as target lesions.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum of diameters. If lymph nodes are to be included in the sum, then, as noted above, only the short axis is added into the sum. The baseline sum of diameters will be used asa reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease), including pathological lymph nodes, should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as “present,” “absent,” or in rare cases “unequivocal progression.”

In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the Case Report Form (CRF) (e.g., “multiple enlarged pelvic lymph nodes” or “multiple liver metastases”).

RESPONSE CRITERIA

a. Evaluation of Target Lesions

This section provides the definitions of the criteria used to determine objective tumor response for target lesions.

• Complete response (CR): disappearance of all target lesions

Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

• Partial response (PR): at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of diameters

• Progressive disease (PD): at least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (nadir), including baseline

In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

The appearance of one or more new lesions is also considered progression.

• Stable disease (SD): neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum on study

b. Special Notes on the Assessment of Target Lesions

- **Lymph Nodes.** Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to < 10 mm on study. This means that when lymph nodes are included as target lesions, the sum of lesions may not be zero even if CR criteria are met since a normal lymph node is defined as having a short axis < 10 mm.
- **Target Lesions That Become Too Small to Measure.** While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). However, sometimes lesions or lymph nodes that are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being too small to measure. When this occurs, it is important that a value be recorded on the CRF as follows:

  - If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm.

  - If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned and BML (below measurable limit) should be ticked. (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well and BML should also be ticked.)

  However, to reiterate, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm, and, in that case, BML should not be ticked.
- **Lesions That Split or Coalesce on Treatment.** When non-nodal lesions fragment, the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such

that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the coalesced lesion.

c. Evaluation of Non-Target Lesions

This section provides the definitions of the criteria used to determine the tumor response for the group of non-target lesions. Although some non-target lesions may actually be measurable, they need not be measuredand, instead, should be assessed only qualitatively at the timepoints specified in the protocol.

• CR: disappearance of all non-target lesions and (if applicable) normalization of tumor marker level)

All lymph nodes must be non-pathological in size (< 10 mm short axis).

• Non-CR/Non-PD: persistence of one or more non-target lesion(s) and/or (if applicable) maintenance of tumor marker level above the normal limits

• PD: unequivocal progression of existing non-target lesions

The appearance of one or more new lesions is also considered progression.

d. Special Notes on Assessment of Progression of Non-Target Disease

- **When the Patient Also Has Measurable Disease.** In this setting, to achieve unequivocal progression on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease in a magnitude that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest increase in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.
- **When the Patient Has Only Non-Measurable Disease.** This circumstance arises in some Phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above; however, in this instance, there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable), a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease; that is, an increase in tumor burden representing an additional 73% increase in volume (which is equivalent to a 20% increase in diameter in a measurable lesion). Examples include an increase in a pleural effusion from “trace” to “large” or an increase in lymphangitic disease from localized to widespread or may be described in protocols as “sufficient to require a change in therapy.” If unequivocal progression is seen, the patient should be considered to have had overall PD at that point. Although itwould be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so; therefore, the increase must be substantial.
- **When the patient has bone lesions at baseline.** When a bone scan is the sole indicator of progression, progression in bone will be defined as when at least two or more new lesions are seenon bone scan compared with screening. In situations where the scan findings are suggestive of a flarereaction or apparent new lesion(s) that may represent trauma, these results must be confirmed with other imaging modalities such as MRI or fine-cut CT to constitute progression. Only a single new bone lesion on bone scan is required for progression if the lesion can be correlated on CT or MRI.

e. New Lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal, that is, not attributable to differences in scanning technique, change in imaging modality, or findings thought to represent something other than tumor (for example, some "new" bone lesions may be simply healing or flare of preexisting lesions). This is particularly important when the patient's baseline lesions show PR or CR. For example, necrosis of a liver lesion may be reported on a CT scan report as a "new" cystic lesion, which it is not.

A lesion identified during the study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-upevaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

New osteoblastic bone lesions identified on plain films, CT, or MRI will not be considered progression in an otherwise stable or responding subject if, in the opinion of the physician, the osteoblastic lesion appears to be healing or a response to therapy.

EVALUATION OF RESPONSE

a. Timepoint Response (Overall Response)

It is assumed that at each protocol-specified timepoint, a response assessment occurs. Table 12 provides a summary of the overall response status calculation at each timepoint for patients who have measurable disease at baseline.

When patients have non-measurable (therefore non-target) disease only, Table 13 is to be used.

Table 12 Timepoint Response: Patients with Target Lesions (with or without Non-Target Lesions)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

CR = complete response; NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease.

Table 13 Timepoint Response: Patients with Non-Target Lesions Only

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD a
Not all evaluated	No	NE
Unequivocal PD	Yes or no	PD
Any	Yes	PD

CR = complete response; NE = not evaluable; PD = progressive disease.
[a] "Non-CR/non-PD" is preferred over "stable disease" for non-target disease since stable disease is increasingly used as an endpoint for assessment of efficacy in some trials; thus, assigning "stable disease" when no lesions can be measured is not advised.

b. Missing Assessments and Not-Evaluable Designation

When no imaging/measurement is done at all at a particular timepoint, the patient is not evaluable at that timepoint. If only a subset of lesion measurements are made at an assessment, usually the case is also considered not evaluable at that timepoint, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned timepoint response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50 mm with three measured lesions and, during the study, only two lesions were assessed, but those gave a sum of 80 mm; the patient will have achieved PD status, regardless of the contribution of the missing lesion.

If one or more target lesions were not assessed either because the scan was not done or the scan could not be assessed because of poor image quality or obstructed view, the response for target lesions should be "unable to assess" since the patient is not evaluable. Similarly, if one or more non-target lesions are not assessed, the response for non-target lesions should be "unable to assess" except where there is clear progression. Overall response would be "unable to assess" if either the target response or the non-target response is "unable to assess," except where this is clear evidence of progression as this equates with the case being not evaluable at that timepoint.

Tab 14 Best Overall Response When Confirmation Is Required

Overall Response at First Timepoint	Overall Response at Subsequent Timepoint	Best Overall Response
CR	CR	CR
CR	PR	SD, PD, or PR [a]
CR	SD	SD, provided minimum duration for SD was met; otherwise, PD
CR	PD	SD, provided minimum duration for SD was met; otherwise, PD
CR	NE	SD, provided minimum duration for SD was met; otherwise, NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD, provided minimum duration for SD was met; otherwise, PD
PR	NE	SD, provided minimum duration for SD was met; otherwise, NE
NE	NE	NE

CR = complete response; NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease.

[a] If a CR is truly met at the first timepoint, any disease seen at a subsequent timepoint, even disease meeting PR criteria relative to baseline, qualifies as PD at that point (since disease must have reappeared after CR). Best response would depend on whether the minimum duration for SD was met. However, sometimes CR may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR,at the first timepoint. Under these circumstances, the original CR should be changed to PR and the best response is PR.

c. Special Notes on Response Assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to "normal" size (< 10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of thenodes. As noted earlier, this means that patients with CR may not have a total sum of "zero" on the CRF.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response; it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target diseaseas shown in Tables 9-12.

For equivocal findings of progression (e.g., very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment progression is confirmed, the date of progression should be the earlier date when progression was suspected.

If a patient undergoes an excisional biopsy or other appropriate approach (e.g., multiple passes with large core needle) of a new lesion or an existing solitary progressive lesion that following serial sectioning and pathological examination reveals no evidence of malignancy (e.g., inflammatory cells, fibrosis, etc.), then the new lesion or solitary progressive lesion will not constitute disease progression.

In studies for which patients with advanced disease are eligible (i.e., primary disease still or partially present), the primary tumor should also be captured as a target or non-target lesion, as appropriate. This is to avoid an incorrect assessment of CR if the primary tumor is still present but not evaluated as a target or non-target lesion.

APPENDIX IV: Preexisting Autoimmune Diseases

Subjects should be carefully questioned regarding their history of acquired or congenital immune deficiencies or autoimmune disease. Subjects with any history of immune deficiencies or autoimmune disease listed in the table below are excluded from participating in the study. Possible exceptions to this exclusion could be subjects with a medical history of such entities as atopic disease or childhood arthralgias where the clinical suspicion of autoimmune disease is low. Patients with a history of autoimmune-related hypothyroidism on a stable dose of thyroid replacement hormone may be eligible for this study. In addition, transient autoimmune manifestations of an acute infectious disease that resolved upon treatment of the infectious agent are not excluded (e.g., acute Lyme arthritis). Please contact the Sponsor regarding any uncertainty over autoimmune exclusions.

Autoimmune Diseases and Immune Deficiencies

Acute disseminated encephalomyelitis Addison's disease Ankylosing spondylitis Antiphospholipid antibody syndrome Aplastic anemia Autoimmune hemolytic anemia Autoimmune hepatitis Autoimmune hypoparathyroidism Autoimmune hypophysitis Autoimmune myocarditis Autoimmune oophoritis Autoimmune orchitis Autoimmune thrombocytopenic purpura Behcet's disease Bullous pemphigold Chronic inflammatory demyelinating polyneuropathy Chung-Strauss syndrome Crohn's disease	Dermatomyositis Diabetes mellitus Type 1 Dysautonomia Epidermolysis bullosa acquista Gestational pemphigoid Giant cell arteritis Goodpasture's syndrome Graves' disease Guillain-Barré syndrome Hashimoto's disease IgA nephropathy Inflammatory bowel disease Interstitial cystitis Kawasaki's disease Lambert-Eaton myasthenia syndrome Lupus erythematosus Lyme disease - chronic Mooren's ulcer Morphea Multiple sclerosis Myasthenia gravis	Neuromyotonia Opsoclonus myoclonus syndrome Optic neuritis Ord's thyroiditis Pemphigus Pernicious anemia Polyarteritis nodusa Polyarthritis Polyglandular autoimmune syndrome Primary biliary cirrhosis Psoriasis Reiter's syndrome Rheumatoid arthritis Sarcoidosis Scleroderma Sjögren's syndrome Stiff-Person syndrome Takayasu's arteritis Ulcerative colitis Vogt-Kovanagi-Harada disease Granulomatosis with polyangiitis

APPENDIX V: Eastern Cooperative Oncology Group Performance Status Scale

Eastern Cooperative Oncology Group Performance Status Scale

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 housework or office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about > 50% of waking hours
3	Capable of only limited self-care, confined to a bed or chair > 50% of waking hours
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair
5	Dead

APPENDIX VI : Patients Reported Outcomes

European Organisation for Research and Treatment of Cancer 30-Item Quality of life Questionnaire

ITALIAN

EORTC QLQ-C30 (version 3.0)

Con questo questionario vorremmo sapere alcune cose su di Lei e sulla Sua salute. La preghiamo di rispondere a tutte le domande ponendo un cerchio attorno al numero che meglio corrisponde alla Sua risposta. Non esiste una risposta "giusta" o "sbagliata". Le Sue informazioni verranno tenute strettamente riservate.

Per favore scriva solo le iniziali del Suo nome e cognome: ⌊ ⌋⌊ ⌋⌊ ⌋

Data di nascita (g, m, a): ⌊ ⌋⌊ ⌋ ⌊ ⌋⌊ ⌋ ⌊ ⌋⌊ ⌋

La data di oggi (g, m, a): 31 ⌊ ⌋⌊ ⌋ ⌊ ⌋⌊ ⌋ ⌊ ⌋⌊ ⌋

	No	Un po'	Parec-chio	Moltis-simo
1. Ha difficoltà nel fare lavori faticosi, come sollevare una borsa della spesa pesante o una valigia?	1	2	3	4
2. Ha difficoltà nel fare una lunga passeggiata?	1	2	3	4
3. Ha difficoltà nel fare una breve passeggiata fuori casa?	1	2	3	4
4. Ha bisogno di stare a letto o su una sedia durante il giorno?	1	2	3	4
5. Ha bisogno di aiuto per mangiare, vestirsi, lavarsi o andare in bagno?	1	2	3	4

Durante gli ultimi sette giorni:	No	Un po'	Parec-chio	Moltis-simo
6. Ha avuto limitazioni nel fare il Suo lavoro o i lavori di casa?	1	2	3	4
7. Ha avuto limitazioni nel praticare i Suoi passatempi-hobby o altre attività di divertimento o svago?	1	2	3	4
8. Le è mancato il fiato?	1	2	3	4
9. Ha avuto dolore?	1	2	3	4
10. Ha avuto bisogno di riposo?	1	2	3	4
11. Ha avuto difficoltà a dormire?	1	2	3	4
12. Ha sentito debolezza?	1	2	3	4
13. Le è mancato l'appetito?	1	2	3	4
14. Ha avuto un senso di nausea?	1	2	3	4
15. Ha vomitato?	1	2	3	4

Continuare alla pagina successiva

ITALIAN

Durante gli ultimi sette giorni:	**No**	**Un po'**	**Parec-chio**	**Moltis-simo**
16. Ha avuto problemi di stitichezza?	1	2	3	4
17. Ha avuto problemi di diarrea?	1	2	3	4
18. Ha sentito stanchezza?	1	2	3	4
19. Il dolore ha interferito con le Sue attività quotidiane?	1	2	3	4
20. Ha avuto difficoltà a concentrarsi su cose come leggere un giornale o guardare la televisione?	1	2	3	4
21. Si è sentito(a) teso(a)?	1	2	3	4
22. Ha avuto preoccupazioni?	1	2	3	4
23. Ha avuto manifestazioni di irritabilità?	1	2	3	4
24. Ha avvertito uno stato di depressione?	1	2	3	4
25. Ha avuto difficoltà a ricordare le cose?	1	2	3	4
26. Le Sue condizioni fisiche o il Suo trattamento medico hanno interferito con la Sua vita familiare?	1	2	3	4
27. Le Sue condizioni fisiche o il Suo trattamento medico hanno interferito con le Sue attività sociali?	1	2	3	4
28. Le Sue condizioni fisiche o il Suo trattamento medico Le hanno causato difficoltà finanziarie?	1	2	3	4

Per le seguenti domande ponga un cerchio intorno al numero da 1 a 7 che meglio corrisponde alla Sua risposta

29. Come valuterebbe in generale la Sua salute durante gli ultimi sette giorni?

1	2	3	4	5	6	7
Pessima						Ottima

30. Come valuterebbe in generale la Sua qualità di vita durante gli ultimi sette giorni?

1	2	3	4	5	6	7
Pessima						Ottima

European Organisation for research and treatment of cancer for colorectal cancer

ITALIAN

EORTC QLQ – CR29

Talvolta i pazienti accusano i seguenti sintomi. La preghiamo di indicare il grado con cui ha provato questi sintomi durante gli ultimi sette giorni. Risponda tracciando un cerchio intorno al numero che meglio definisce la Sua situazione.

Durante la settimana scorsa:	**No**	**Un po'**	**Parec-chio**	**Moltis-simo**
31. Ha urinato spesso durante il giorno?	1	2	3	4
32. Ha urinato spesso durante la notte?	1	2	3	4
33. Ha subito una perdita involontaria di urina?	1	2	3	4
34. Ha provato dolore nell'urinare?	1	2	3	4
35. Ha provato dolore addominale?	1	2	3	4
36. Ha provato dolori alle natiche o alla zona dell'ano o del retto?	1	2	3	4
37. Ha avvertito una sensazione di gonfiore all'addome?	1	2	3	4
38. Ha trovato sangue nelle feci?	1	2	3	4
39. Ha trovato muco nelle feci?	1	2	3	4
40. Ha provato secchezza alla bocca?	1	2	3	4
41. Ha perduto i capelli in seguito alla terapia?	1	2	3	4
42. Ha riscontrato problemi con il senso del gusto?	1	2	3	4

Durante la settimana scorsa:	**No**	**Un po'**	**Parec-chio**	**Moltis-simo**
43. Ha avuto preoccupazioni per la Sua salute futura?	1	2	3	4
44. Ha avuto preoccupazioni riguardo al peso?	1	2	3	4
45. Si è sentito/a fisicamente meno attraente in conseguenza della malattia o della terapia?	1	2	3	4
46. Si è sentito/a meno virile/femminile in conseguenza della malattia o della terapia?	1	2	3	4
47. Si è sentito/a insoddisfatto/a del Suo corpo?	1	2	3	4
48. Ha una sacca per stomia (colostomia/ileostomia)? (cerchiare la risposta corretta)	Sì		No	

Continuare alla pagina successiva

ITALIAN

Durante la settimena scorsa:	**No**	**Un po'**	**Parec-chio**	**Moltis-simo**
Rispondere a queste domande SOLO SE SI UTILIZZA UNA SACCA PER STOMIA, altrimenti passare oltre:				
49. Ha riscontrato perdite involontarie di gas o flatulenze dalla sacca per stomia?	1	2	3	4
50. Ha riscontrato perdite di feci dalla sacca per stomia?	1	2	3	4
51. La pelle intorno allo stoma si è irritata?	1	2	3	4
52. Ha dovuto cambiare la sacca molto spesso durante il giorno?	1	2	3	4
53. Ha dovuto cambiare la sacca molto spesso durante la notte?	1	2	3	4
54. Ha provato imbarazzo riguardo allo stoma?	1	2	3	4
55. Ha avuto problemi nell'occuparsi della sacca per stomia?	1	2	3	4

Rispondere a queste domande SOLO SE NON SI UTILIZZA UNA SACCA PER STOMIA:				
49. Ha riscontrato perdite involontarie di gas o flatulenze dall'ano?	1	2	3	4
50. Ha riscontrato perdite di feci dall'ano?	1	2	3	4
51. La pelle intorno all'ano si è irritata?	1	2	3	4
52. È andato/a spesso di corpo durante il giorno?	1	2	3	4
53. È andato/a spesso di corpo durante la notte?	1	2	3	4
54. Ha provato imbarazzo nell'andare di corpo?	1	2	3	4

Durante le ultime 4 settimane:	**No**	**Un po'**	**Parec-chio**	**Moltis-simo**
Solo per gli uomini:				
56. In che misura ha provato interesse per il sesso?	1	2	3	4
57. Ha incontrato difficoltà a ottenere o mantenere un'erezione?	1	2	3	4

Solo per le donne:				
58. In che misura ha provato interesse per il sesso?	1	2	3	4
59. Ha provato dolore o fastidio durante il rapporto sessuale?	1	2	3	4

EuroQoL 5 Dimensions Health Questionnarie

Questionario sulla Salute

Versione italiana per l'Italia

(Italian version for Italy)

Sotto ciascun argomento, faccia una crocetta sulla casella (UNA SOLA) che descrive meglio la sua salute OGGI.

CAPACITÀ DI MOVIMENTO

Non ho difficoltà nel camminare ❑
Ho lievi difficoltà nel camminare ❑
Ho moderate difficoltà nel camminare ❑
Ho gravi difficoltà nel camminare ❑
Non sono in grado di camminare ❑

CURA DELLA PERSONA

Non ho difficoltà nel lavarmi o vestirmi ❑
Ho lievi difficoltà nel lavarmi o vestirmi ❑
Ho moderate difficoltà nel lavarmi o vestirmi ❑
Ho gravi difficoltà nel lavarmi o vestirmi ❑
Non sono in grado di lavarmi o vestirmi ❑

ATTIVITÀ ABITUALI (*per es. lavoro, studio, lavori domestici,attività familiari o di svago)*

Non ho difficoltà nello svolgimento delle attività abituali ❑
Ho lievi difficoltà nello svolgimento delle attività abituali ❑
Ho moderate difficoltà nello svolgimento delle attività abituali ❑
Ho gravi difficoltà nello svolgimento delle attività abituali ❑
Non sono in grado di svolgere le mie attività abituali ❑

DOLORE O FASTIDIO

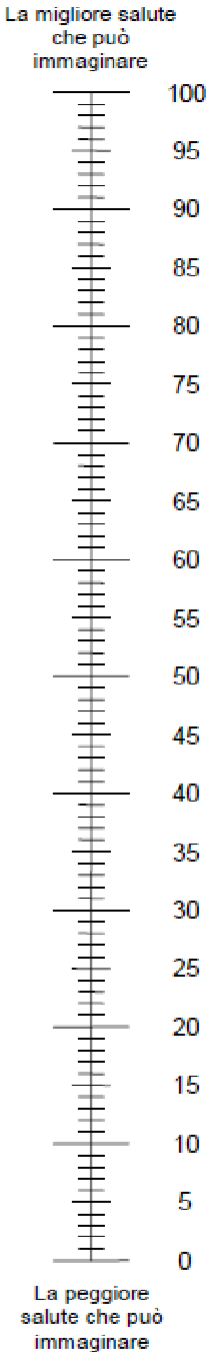
Non provo alcun dolore o fastidio ❑
Provo lieve dolore o fastidio ❑
Provo moderato dolore o fastidio ❑
Provo grave dolore o fastidio ❑
Provo estremo dolore o fastidio ❑

ANSIA O DEPRESSIONE

Non sono ansioso/a o depresso/a ❑
Sono lievemente ansioso/a o depresso/a ❑
Sono moderatamente ansioso/a o depresso/a ❑
Sono gravemente ansioso/a o depresso/a ❑
Sono estremamente ansioso/a o depresso/a ❑

- Vorremmo sapere quanto è buona o cattiva la sua salute OGGI.
- Questa è una scala numerata che va da 0 a 100.
- 100 rappresenta la migliore salute che può immaginare.
  0 rappresenta la peggiore salute che può immaginare.
- Segni una X sul punto della scala per indicare com'è la sua salute OGGI.
- Poi, scriva nella casella qui sotto il numero che ha segnato sulla scala numerata.

LA SUA SALUTE OGGI = ☐



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APPENDIX VII: SAE/AESI FORM

CIOMS FORM

SUSPECT ADVERSE REACTION REPORT	

I. REACTION INFORMATION

1. PATIENT INITIALS (first, last)	1a. COUNTRY	2. DATE OF BIRTH Day	Month	Year	2a. AGE Years	3. SEX	4-6 REACTION ONSET Day	Month	Year	8-12 CHECK ALL APPROPRIATE TO ADVERSE REACTION
7 + 13 DESCRIBE REACTION(S) (including relevant tests/lab data)										☐ PATIENT DIED ☐ INVOLVED OR PROLONGED INPATIENT HOSPITALISATION ☐ INVOLVED PERSISTENCE OR SIGNIFICANT DISABILITY OR INCAPACITY ☐ LIFE THREATENING

II. SUSPECT DRUG(S) INFORMATION

14. SUSPECT DRUG(S) (include generic name)		20 DID REACTION ABATE AFTER STOPPING DRUG? ☐ YES ☐ NO ☐ NA
15. DAILY DOSE(S)	16. ROUTE(S) OF ADMINISTRATION	21. DID REACTION REAPPEAR AFTER REINTRODUCTION? ☐ YES ☐ NO ☐ NA
17. INDICATION(S) FOR USE		
18. THERAPY DATES (from/to)	19. THERAPY DURATION	

III. CONCOMITANT DRUG(S) AND HISTORY

22. CONCOMITANT DRUG(S) AND DATES OF ADMINISTRATION (exclude those used to treat reaction)
23. OTHER RELEVANT HISTORY (e.g. diagnostics, allergics, pregnancy with last month of period, etc.)

IV. MANUFACTURER INFORMATION

24a. NAME AND ADDRESS OF MANUFACTURER		
	24b. MFR CONTROL NO.	
24c. DATE RECEIVED BY MANUFACTURER	24d. REPORT SOURCE ☐ STUDY ☐ LITERATURE ☐ HEALTH PROFESSIONAL	
DATE OF THIS REPORT	25a. REPORT TYPE ☐ INITIAL ☐ FOLLOWUP	

APPENDIX VIII: Management Algorithms For Nivolumab And Ipilimumab

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

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

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

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

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

GI Adverse Event Management Algorithm

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

Grade of Diarrhea/ Colitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Diarrhea:< 4 stools/day over baseline; Colitis: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hrs; not interfering with ADL Colitis: abdominal pain; blood in stool	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as grade 3/4
Grade 3-4 Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; interfering with ADL Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

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

Renal Adverse Event Management Algorithm

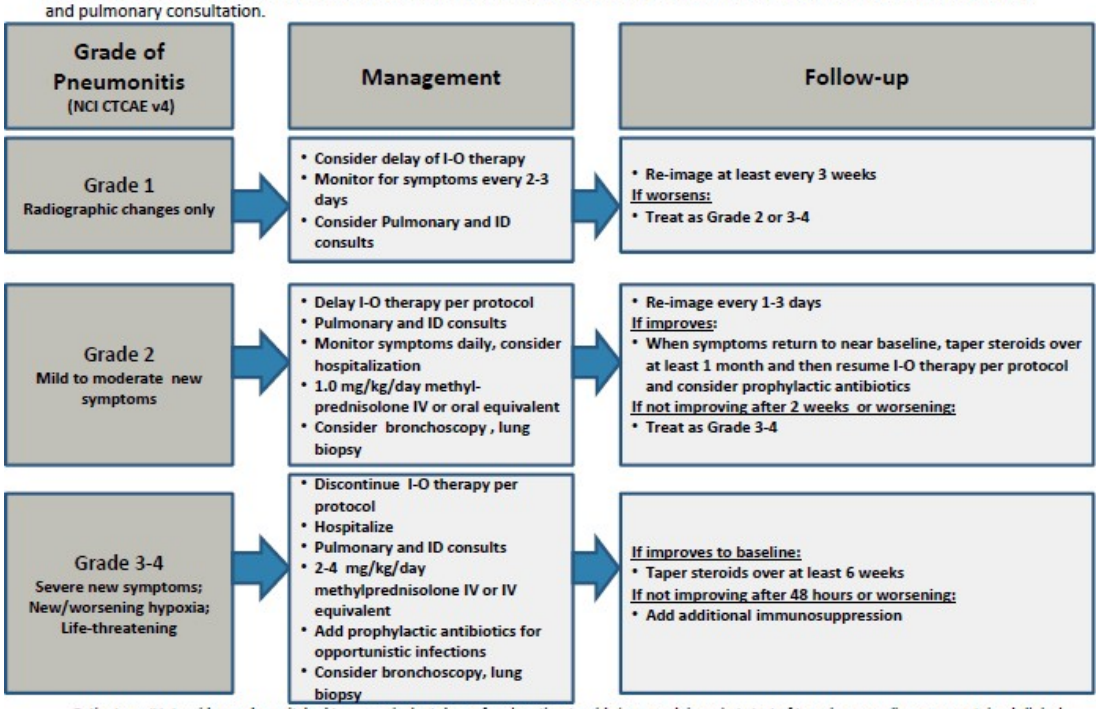
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy

Grade of Creatinine Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 Creatinine > ULN and > than baseline but ≤ 1.5x baseline	• Continue I-O therapy per protocol • Monitor creatinine weekly	<u>If returns to baseline</u>: •Resume routine creatinine monitoring per protocol <u>If worsens:</u> •Treat as Grade 2 or 3/4
Grade 2-3 Creatinine > 1.5x baseline to ≤ 6x ULN	• Delay I-O therapy per protocol • Monitor creatinine every 2-3 days • 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider renal biopsy with nephrology consult	<u>If returns to Grade 1:</u> •Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol <u>If elevations persist > 7 days or worsen:</u> •Treat as Grade 4
Grade 4 Creatinine > 6x ULN	• Discontinue I-O therapy per protocol • Monitor creatinine daily • 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent • Consult nephrologist • Consider renal biopsy	<u>If returns to Grade 1 :</u> Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

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

Pulmonary Adverse Event Management Algorithm

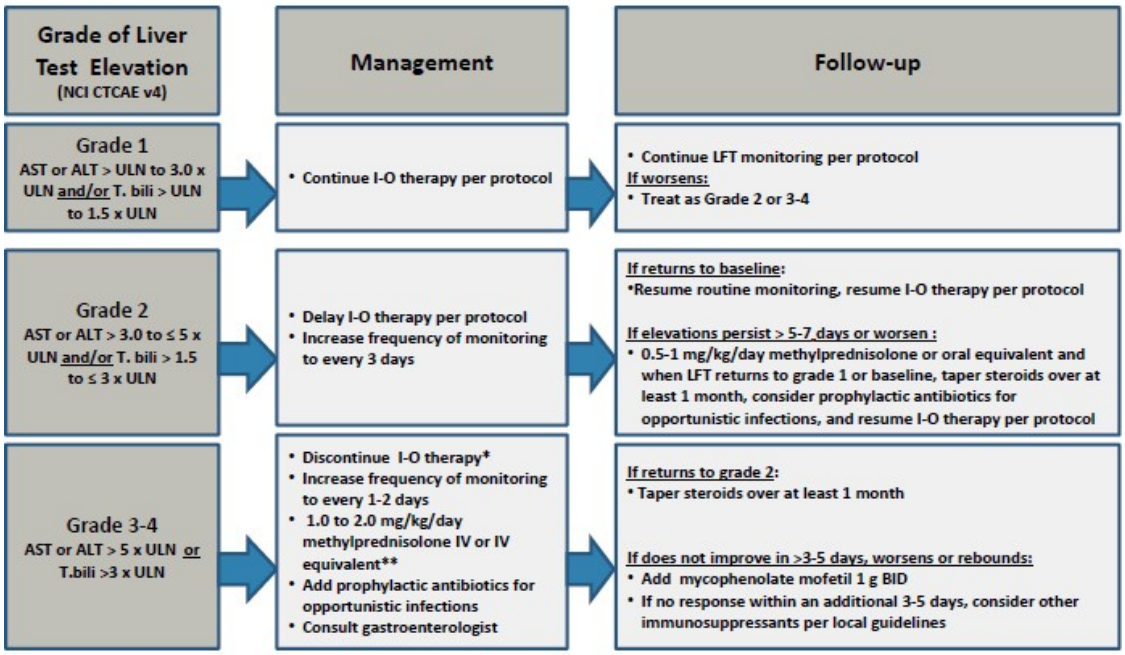
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

Hepatic Adverse Event Management Algorithm

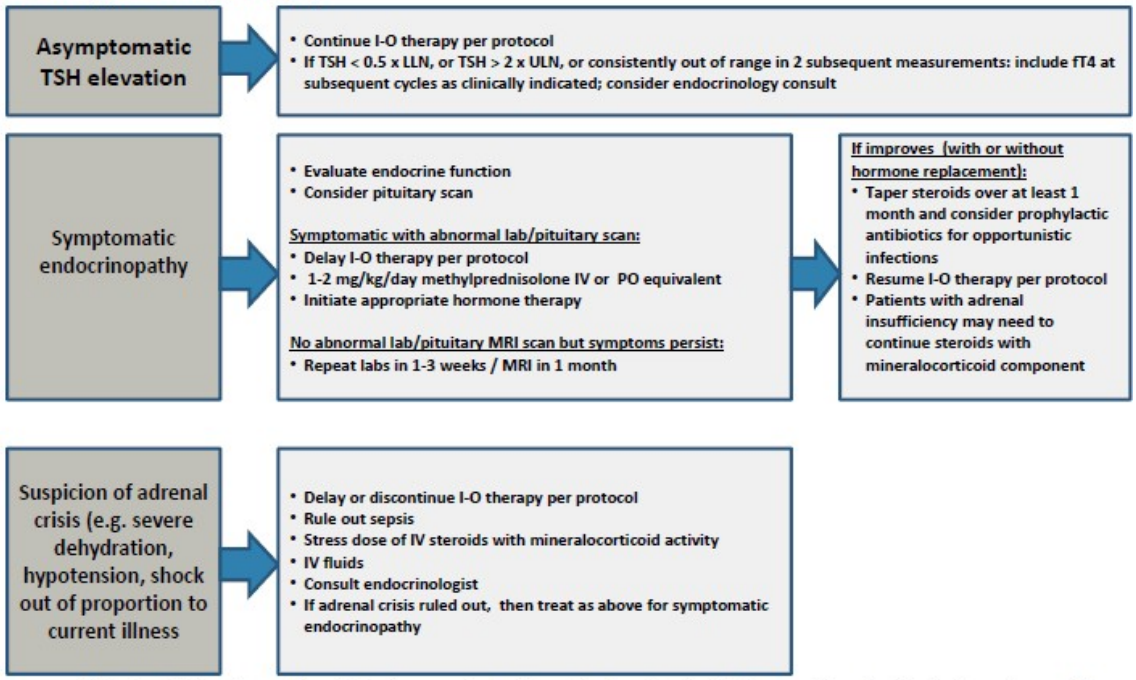
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN or T.bili ≤ 5 x ULN.
**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Endocrinopathy Management Algorithm

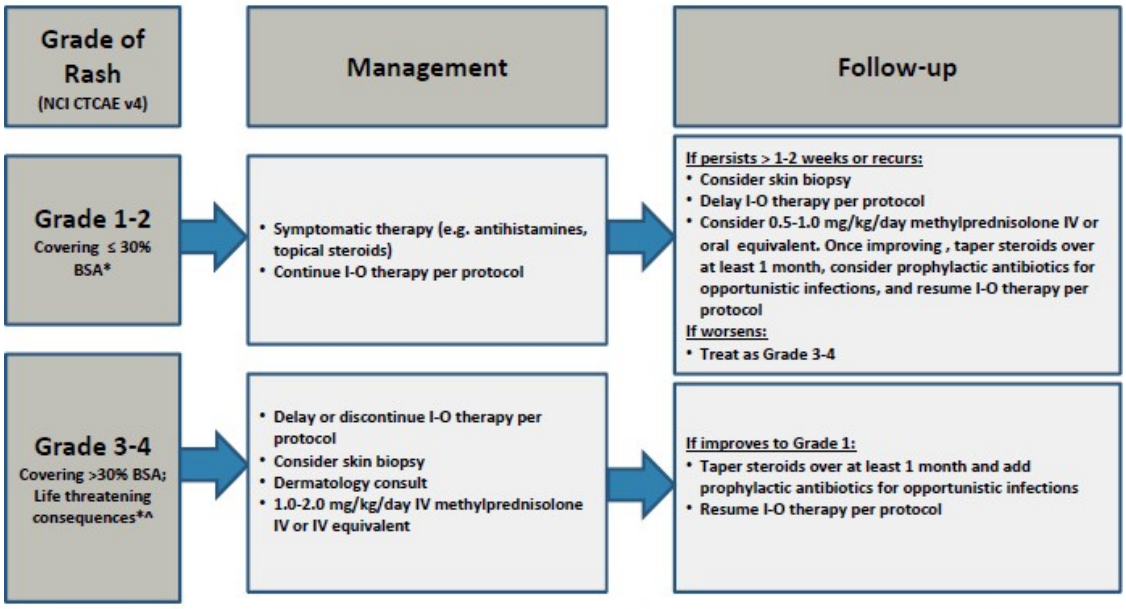
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

Skin Adverse Event Management Algorithm

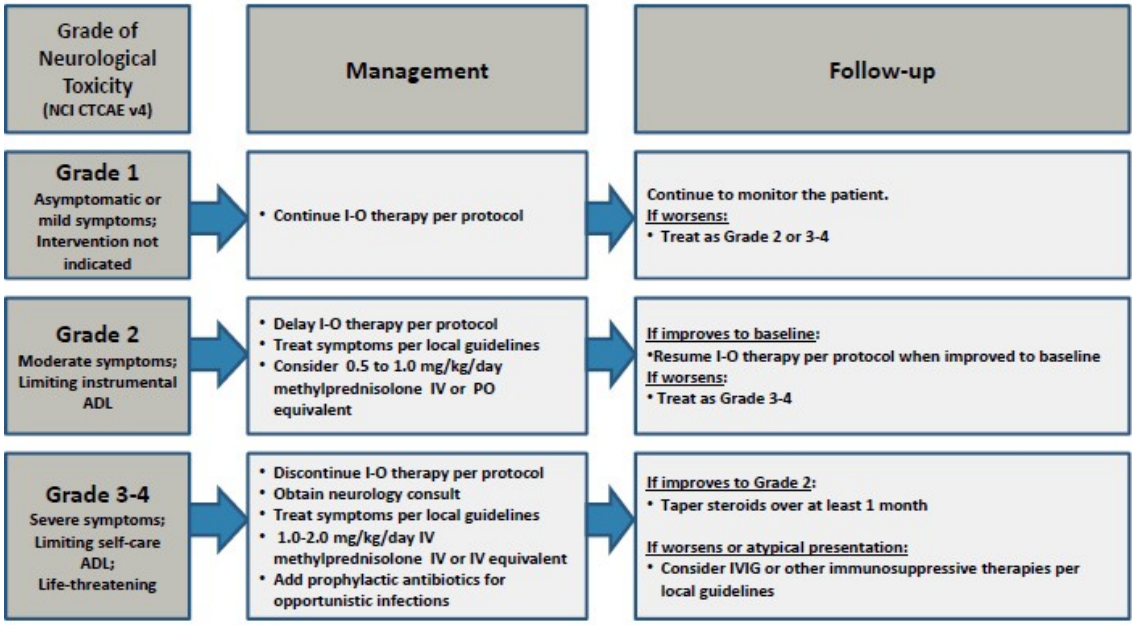
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.
^If SJS/TEN is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

Neurological Adverse Event Management Algorithm

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



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