Clinical Research Protocol

A phase II, open-label trial of nivolumab, ipilimumab, and hypofractionated radiotherapy in adults with newly diagnosed, *MGMT* unmethylated glioblastoma

Regulatory Sponsor/Overall Principal Investigator: Sylvia Kurz, MD, PhD
Laura and Isaac Perlmutter Cancer Center
NYU School of Medicine
240 East 38th Street, 19th Floor
New York, NY 10016
Tel: 212-731-6267
Fax: 646-754-9696
Email: Sylvia.Kurz@nyulangone.org

Funding Provider: Bristol-Myers Squibb (BMS)

Study Products: Nivolumab (BMS-936558)
Ipilimumab (BMS-734016)

NYU Protocol Number: s17-00218

BMS Study ID: CA209-9JE

IND Number: 136853

NCT03367715

Co-Investigators:

Joshua S. Silverman, MD, PhD
Department of Radiation Oncology, NYU School of Medicine
Email: Joshua.S.Silverman@nyulangone.org

Erik P. Sulman, MD, PhD
Department of Radiation Oncology, NYU School of Medicine
Email: Erik.Sulman@nyulangone.org

Douglas Kondziolka, MD
Department of Neurosurgery, NYU School of Medicine
Email: Douglas.Kondziolka@nyulangone.org

Matija Snuderl, MD
Molecular Pathology Division, Department of Pathology
NYU School of Medicine
Email: Matija.Snuderl@nyulangone.org

Biostatistician:

Andrea B. Troxel, ScD
Division of Biostatistics, Department of Population Health
NYU School of Medicine
Email: Andrea.Troxel@nyulangone.org

Medical Monitor:

Benjamin Cooper, MD
Department of Radiation Oncology, NYU School of Medicine
Email: Benjamin.Cooper@nyulangone.org

Initial version: 11/29/2017
Amended: 1/4/18
11/5/18
6/20/2019
11/19/2019
5/29/2020

STUDY SYNOPSIS

Protocol Title: A phase II, open-label, trial of nivolumab, ipilimumab, and hypofractionated radiotherapy in adults with newly diagnosed, *MGMT* unmethylated glioblastoma

NYU Protocol Number: s17-00218

BMS Study ID: CA209-9JE

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Principal Investigator/Sponsor: Sylvia Kurz, MD, PhD

Study Site: NYU School of Medicine (NYUSoM)

Investigational Products, Dose and Mode of Administration, Duration of Treatment with Investigational Products:

- Nivolumab 3 mg/kg IV every 2 weeks for up to 2 years.
- Ipilimumab 1 mg/kg IV every 6 weeks for up to 2 years.
- Radiotherapy to a total dose of 45 Gy, given in 15 consecutive fractions of 3 Gy each fraction

Study Phase: II

Research Hypothesis: This study will test the hypothesis that hypofractionated radiotherapy (45 Gy in 15 fractions) will result in reduced treatment-induced severe lymphopenia and immunosuppression compared to standard chemoradiation and enhance the efficacy of combination immune checkpoint therapy to improve the overall survival of newly diagnosed *MGMT*-unmethylated glioblastoma (GBM) subjects.

Objectives:

Primary

- **Main Study Cohort:** To estimate the overall survival at 1 year (1-yr OS) of adults with newly diagnosed, MGMT unmethylated GBM administered nivolumab, ipilimumab and hypofractionated RT.

- **Surgical Study Cohort:** To assess Safety, Feasibility, and Tolerability of neoadjuvant ipilimumab and nivolumab (prior to tumor re-resection), and estimate rate of T-cell pathway induction.

Secondary

- **Main Study Cohort**
    - Assess safety and tolerability
    - Estimate additional measures of efficacy, including 2-year OS, median OS, median PFS, radiographic response rate, and median duration of response.
- **Surgical Study Cohort**
    - Estimate efficacy measures including 1-yr OS, 2-year OS, median OS, median PFS, radiographic response rate, and median duration of response.

Exploratory

- Assess association of 1-yr OS with: tumor tissue biomarkers including mutation burden, neoantigen load, immune marker expression [e.g. PD-L1, lymphocyte activation gene 3 (LAG3), indoleamine-2,3-dioxygenase (IDO) and T-cell immunoglobulin and mucin domain 3 (TIM3)], and PD-1+ tumor infiltrating lymphocytes (TILs).

- Assess association of 1-yr OS with baseline and change at 6 weeks in levels of circulating biomarkers, including: regulatory T cells (T_{reg}); myeloid-derived suppressor cells (MDSC); and immune cell populations as profiled by high-parameter flow cytometry that simultaneously quantifies expression of proteins related to checkpoint inhibitors (PD1, CTLA4, TIM3, TIGIT, 41BB, CD150, LAG3, BTLA), differentiation status (CD45RA, CCR7, CD57, CD95, CD127), and other traits (CD25, IL12R, CXCR3, CXCR6).

- Evaluate effect of study regimen on total lymphocyte counts (TLC) and CD4 counts at fixed timepoints for up to 1 year compared to baseline.

- Explore whether sodium MRI, a non-invasive surrogate measure of cell death in tissue, can differentiate between treatment-related cell death/necrosis ("pseudoprogression") versus tumor progression and serve as a potential non-invasive pharmacodynamic biomarker.

Study Design:

This is an open-label, phase II trial of nivolumab, ipilimumab and hypofractionated radiation therapy in adult patients with newly diagnosed, *MGMT* unmethylated GBM (confirmed centrally at NYUSoM) with the primary objective of determining the overall survival at 1 year.

Safety Lead-In: The study will start with a Safety Lead-In consisting of the first 6 subjects (Section 6.1) to further define the safety and tolerability of nivolumab and ipilimumab when given in combination with hypofractionated radiotherapy in previously untreated, newly diagnosed GBM patients with unmethylated *MGMT* promoter. Note, the patients enrolled in the Safety Lead-In cohort will subsequently be analyzed in the Main Study Cohort (see below). Also note, special safety provisions are included for the first 3 subjects enrolled (see Section 6.1.1). After the first 6 subjects are enrolled, enrollment will stop and subjects will be observed for 40 days for dose-limiting toxicities (DLTs). After all subjects in the Safety Lead-In cohort have completed the DLT observation period, the safety data will be reviewed by the Sponsor/Overall PI and the NYU DSMC. If 2 or more of the first 6 subjects in the Safety Lead-In experiences a DLT during the 28 day DLT observation period, the sponsor, with consultation from BMS and the FDA, may redesign the dosing and schedule of the study treatment or will stop the study for unacceptable toxicity. If 1 or fewer of the first 6 subjects experiences a DLT at the end of the 28 day DLT observation period, the study will continue enrollment onto the phase II part of the study.

Main Study Cohort: The Phase II trial is an open-label, one-stage phase II study and will enroll a total of 24 subjects, which includes the 6 subjects in the Safety Lead-In. A Bayesian monitoring approach will be applied to assess safety throughout the Phase II trial with pre-defined stopping rules.

Surgical Study Cohort: The Surgical Study Cohort will open once the Safety Lead-In is completed. We will use a single-stage design and enroll up to 16 subjects with newly diagnosed MGMT-unmethylated glioblastoma who are amenable to a larger resection will be included in this cohort.

Study Procedures:

After screening procedures and registration, within 6 weeks of the first diagnostic surgery for glioblastoma, all subjects will initiate study treatment with one dose of nivolumab 3 mg/kg and one dose of ipilimumab 1mg/kg.

- Patients in the **Safety Lead-In**/**Main Study Cohort** will begin hypofractionated radiotherapy (HFRT) within 1 week of first nivolumab and ipilimumab dose.
- Patients in the **Surgical Study Cohort** (opens once Safety Lead-In completed) will undergo craniotomy and tumor re-resection within 2 weeks from the first dose of nivolumab and ipilimumab and begin HFRT 3 weeks following surgery

All subjects will receive HFRT to a total dose of 45 Gy, given in 15 consecutive fractions of 3 Gy each fraction.

All subjects will continue to receive nivolumab 3 mg/kg IV every 2 weeks and ipilimumab 1 mg/kg every 6 weeks from day of first nivolumab and ipilimumab administration. One treatment cycle will be defined as 42 days (6 weeks), corresponding to 3 doses of nivolumab and one dose of ipilimumab. Nivolumab and ipilimumab administration will continue for 2 years or until progression of disease, unacceptable toxicity, death, or another protocol criterion for subject withdrawal is met, whichever comes first.

Subjects will be evaluated every 8 weeks with radiographic imaging to assess response to treatment. The primary efficacy endpoint will be overall survival at 1 year. Disease assessments on study will be made using the Response Assessment for Neuro-Oncology (RANO) criteria. However, non-traditional Immunotherapy Response Assessment for Neuro-Oncology (iRANO) criteria may be considered for patient management. Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

End of treatment assessments will be performed within 7 days after last drug administration or within 7 days after decision to end treatment. Site visits and safety laboratories are to be performed at 30 days (±7 days) and 90 days (±14 days) after the last study drug is given, unless the subject is unable to travel due to deteriorating medical condition, due to the potential risk for delayed immune-related toxicities. Post-treatment, all participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 30 days of last study drug. Assessments may continue for ongoing reportable adverse events.

Subjects who discontinue study drug must continue to be followed for collection of outcome and/or survival follow-up data until death, withdrawal of permission to record at least survival data, or subject is lost to follow-up.

Following the 90-day post-drug visit, all subjects will be contacted every 3 months (±14 days) to assess for study-treatment related toxicities, initiation of any new anti-cancer treatments, and survival status. Contact may be performed by a site visit, telephone contact, e-mail, or mail.

Study Population:

Inclusion Criteria:

1. Male or female subjects aged ≥18 years.

2. Histopathological evidence of glioblastoma or gliosarcoma, WHO grade IV.
3. Tumor *MGMT* promoter DNA not methylated (i.e., unmethylated) by central testing.
4. There will be to study cohorts:
    a. **Main Study Cohort:** Maximal tumor diameter (including residual tumor and resection cavity if subjects had tumor resection rather than only stereotactic biopsy) of 6.6 cm or less. Maximal tumor size allowed is derived from an estimated maximal radiotherapy planning target volume (PTV) of 150 cm^3. Note, the maximal tumor diameter for the first 3 subjects enrolled will be 5 cm.
    b. **Surgical Study Cohort:** Patients who received tumor biopsy or subtotal tumor resection and are amenable to a second tumor resection with expected post-operative tumor diameter of ≤ 6 cm (PTV of ≤ 150 cm^3)
5. Subjects must not have received any prior standard or investigational anti-tumor therapy other than surgery and must not intend to receive any standard or investigational anti-tumor therapy other than the study regimen.
6. Karnofsky performance status (Appendix 2) of ≥60.
7. Availability of a paraffin-embedded or frozen tumor-tissue block with a minimum of 1 cm^2 of tumor surface area, or 20 unstained slides from the glioblastoma tissue specimen if a tumor block cannot be submitted. **For Surgical Study Cohort:** availability of pre- and post-operative tumor tissue is required.
8. Subjects must start study agent within 6 weeks from the first diagnostic surgery for glioblastoma.
9. Begin of treatment with study agent:
    - **Main Study Cohort**: Subjects must start study agents no sooner than 2 weeks from last surgical resection and 1 week for stereotactic biopsy.
    - **Surgical Study Cohort:** Subjects must receive 1st dose of study agents 2 weeks prior to tumor re-resection and must resume study agents no sooner than 2 weeks from date of tumor re-resection.
10. A contrast-enhanced MRI must be obtained within 21 days of the first dose of study treatment.
11. Adequate hematologic, hepatic, and renal function defined by
    a. White blood cell count ≥ 2.0 x 10^9/L
    b. Absolute neutrophil count ≥ 1.5 x 10^9/L
    c. Platelet count ≥ 100 x 10^9/L
    d. Hemoglobin > 9 g/dL
    e. Serum creatinine ≤1.5 x upper limit of normal (ULN) or creatinine clearance (CrCl) ≥ 40 mL/min according to the Cockcroft-Gault formula or local institutional standard method
    f. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 3 x ULN
    g. Total bilirubin ≤ 1.5 x ULN (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL)
12. Women of child-bearing potential (WOCBP) and men able to father a child must agree to use highly effective contraception (any contraceptive method with a failure rate of less than 1% per year) while on study drug and for 23 weeks (for women) or 31 weeks (for men) after the last dose of study drug.
    a. WOCBP must have a negative serum or urine pregnancy test within 24 hours of initiation of study drug.

b. WOCBP is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes. In addition, women under the age of 55 must have a documented serum follicle stimulating hormone (FSH) level less than 40 mIU/mL to be defined as post-menopausal.

c. WOCBP receiving nivolumab will be instructed to adhere to contraception for a period of 23 weeks after the last dose of investigational product. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product. These durations have been calculated using the upper limit of the half-life for nivolumab (25 days) and are based on the protocol requirement that WOCBP use contraception for 5 half-lives plus 30 days and men who are sexually active with WOCBP use contraception for 5 half-lives plus 90 days.

d. Highly effective contraceptive measures include: stable use of oral contraceptives such as combined estrogen and progestogen and progestogen only hormonal contraception or other prescription pharmaceutical contraceptives for 2 or more menstrual cycles prior to screening; intrauterine device [IUD]; intrauterine hormone-releasing system (IUS); bilateral tubal ligation; vasectomy and sexual abstinence.

e. Contraception is not required for men with documented vasectomy.

f. Pregnancy testing and contraception are not required for women with documented hysterectomy or tubal ligation.

g. Women must not be breastfeeding.

13. Willing to and capable of providing written informed consent prior to any study related procedures.
14. Ability and willingness to comply with all study requirements, including scheduled visits, treatment plans, laboratory tests, and other study-related procedures.

Exclusion Criteria

15. Prior use of any standard or investigational anti-tumor therapy other than surgery
16. Planned participation in another study of an investigational agent or investigational device or planned use of any other agent or therapeutic device intended for therapy of glioma.
17. Infratentorial tumors.
18. Patients with >1 cm midline shift on postoperative, baseline brain MRI.
19. Diffuse leptomeningeal gliomatosis.
20. Known mutation of the *IDH1* or *IDH2* genes in the tumor, since glioblastomas with these mutations have different biology and are associated with improved prognosis.
    a. Documentation that no *IDH1* or *IDH2* mutations are present in the tumor by a CLIA approved laboratory is required prior to initiation of study treatment.
21. Intracranial hemorrhage grade >1 not attributable to recent neurosurgery.
22. Systemic treatment with either immunosuppressive doses of corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration.
    a. Subjects on a standard high-dose steroid taper after craniotomy or stereotactic biopsy may have received a higher dose of corticosteroids within 14 days of registration,

however must be at a dose < 10 mg daily prednisone or bioequivalent per day within 5 days prior to initiation of study drug.

b. Administration of steroids through a route known to result in a minimal systemic exposure [i.e., intranasal, intraocular, inhaled, topical, or local injection (e.g., intra-articular injection) corticosteroids (<5% of body surface area)] are permitted in the absence of active autoimmune disease.

c. Subjects requiring adrenal replacement with corticosteroids are eligible if the steroids are at doses ≤ 10 mg prednisone or bioequivalent per day in the absence of active autoimmune disease.

d. Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication) are allowed.

23. Active autoimmune disease that might deteriorate when receiving an immuno-stimulatory agent. The following conditions are <u>not exclusions</u> (subjects with the following conditions are permitted):

    a. Patients with diabetes type I, vitiligo, residual hypo- or hyperthyroidism due to autoimmune condition only requiring hormone replacement, or psoriasis not requiring systemic immunosuppressive treatment, or autoimmune conditions not expected to recur in the absence of an external trigger.

24. Prior organ transplantation, including allogeneic stem cell transplantation.

25. Known history of, or any evidence of active, non-infectious pneumonitis within the last 5 years.

26. Known severe (NCI-CTCAE v4.03 Grade 3 or 4) infusion-related allergy or acute hypersensitivity reaction attributed to any monoclonal antibody, any history of anaphylaxis, or uncontrolled asthma (that is, 3 or more features of partially controlled asthma).

27. Unable tolerate an MRI, or have a contraindication to MRI.

28. Active infection requiring systemic therapy.

29. Known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome.

30. Positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus (HCV antibody) indicating acute or chronic infection.

31. Vaccination within 4 weeks of the first dose of study drug and while on trials is prohibited except for administration of inactivated vaccines. Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.

32. Clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke (< 6 months prior to enrollment), myocardial infarction (< 6 months prior to enrollment), unstable angina, congestive heart failure (≥ New York Heart Association Classification Class II), or serious cardiac arrhythmia requiring medication.

33. Patients with another active cancer [excluding basal cell carcinoma, cervical carcinoma in situ or melanoma in situ]. Prior history of other cancer is allowed, as long as there was no active disease within the prior 2 years.

34. All other unstable, severe, or chronic medical or psychiatric conditions including colitis, inflammatory bowel disease, pneumonitis, pulmonary fibrosis, recent (within the past year) or active suicidal ideation or behavior, known alcohol or drug abuse, or laboratory abnormalities that may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study.

Study Assessments:

Efficacy

Efficacy assessment consisting of a contrast-enhanced brain MRI will be performed every 8 weeks on study, ±7 days, until disease progression is documented or treatment is discontinued (whichever occurs later). Response Assessments will be performed on every brain imaging assessment performed on protocol per RANO criteria.

Safety

Subjects will be evaluated for safety if they have received any study drug. Toxicity assessments will be continuous during the treatment phase. Once subjects reach the survival follow-up phase, either in person or documented telephone calls to assess the subject's status are acceptable. Adverse events and laboratory values will be graded according to the NCI-CTCAE version 4.03.

Statistical Considerations:

All subjects who receive at least 1 dose of ipilimumab and nivolumab will be included in the primary analysis (1yr-OS) of the phase II study.

Sample Size:

Main Study Cohort: Based on historical measures, a 12 month (1yr-OS) of 35% will be considered non-promising; an increase in the 1yr-OS to 60% will be considered promising. We will use a single-stage design and enroll 24 patients. This will provide 80% power to detect an increase in the 1-year OS from 35% to 60%, using a 0.05-level test.

Surgical Study Cohort: Based on historical measures, a T-cell pathway induction rate of 33% will be considered non-promising; an increase in the T-cell pathway induction rate to 64% will be considered promising. We will use a single-stage design and enroll 16 patients. This will provide 80% power to detect an increase in the 1-year OS from 33% to 64%, using a 0.05-level test.

Endpoints:

1yr-OS and radiographic response rates will be estimated with exact 95% confidence intervals; with 24 patients, a 95% CI will be no more than 0.4 units wide for the main study cohort. PFS, defined as the time between treatment initiation and first occurrence of disease progression or death, will be censored at last follow-up if the patient remained alive without disease progression. OS will be determined from the time of treatment initiation until the time of death, with OS being censored at last follow-up if the patient remained alive. The Kaplan–Meier curves will be used to summarize PFS and OS and to estimate the medians.

Adverse events will be graded using CTCAE version 4.03. All subjects who initiate therapy (receive at least one dose of ipilimumab and nivolumab and/or radiotherapy) will be evaluated for safety. A Bayesian monitoring approach will be applied to assess safety throughout the Phase II trial with pre-defined stopping rules.

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List of Abbreviations

ADA	Anti-drug antibody
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADR	Adverse drug reaction
AE	Adverse Event
ALP	Alkaline phosphatase
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
BID	Twice Daily
BMS	Bristol-Myers Squibb
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CI	Confidence Interval
CNS	Central Nervous System
CR	Complete Response
CrCl	Creatinine Clearance
CRF	Case Report Form
CRO	Contract Research Organization
CT	Computed-Tomography
CTL	Cytotoxic T cell
CTO	Clinical Trials Office
CXR	Chest X-ray
DLT	Dose Limiting Toxicity
DR	Duration of Response
DSMC	Data and Safety Monitoring Committee
ECG	Electrocardiogram
ECHO	Echocardiogram
FDA	Food and Drug Administration
FFPE	Formalin-fixed, paraffin-embedded
FISH	Fluorescence in situ hybridization
GBM	Glioblastoma
GCPs	Good Clinical Practices
Gy	Gray (unit)
H_1	Histamine H_1 receptor
HCT	Hematocrit
HFRT	Hypofractionated radiotherapy
HGB	Hemoglobin
HGG	High-grade glioma
HR	Hazard Ratio
IB	Investigator's Brochure
IC_{50}	Concentration of 50% Inhibition
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDH	Isocitrate dehydrogenase
IEC	Independent Ethics Committee
irAEs	Immune-related adverse events
iRANO	Immunotherapy Response Assessment for Neuro-Oncology
IRB	Institutional Review Board
I-O	Immuno-Oncology
KPS	Karnofsky Performance Status
LLN	Lower Limit of Normal
mAb	Monoclonal Antibody
MDSC	Myeloid-derived suppressor cell

MG	Malignant Glioma
MHC	Major histocompatibility complex
MMR	Mismatch repair
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03
NSCLC	Non-small cell lung cancer
NYULH	New York University Langone Health
NYUSoM	New York University School of Medicine
OR	Objective Response
ORR	Objective Response Rate
OS	Overall Survival
PBMC	Peripheral blood mononuclear cells
PCC	Perlmutter Cancer Center
PCV	Procarbazine, lomustine (CCNU), vincristine chemotherapy regimen
PD	Progressive Disease
PFS	Progression Free Survival
PK	Pharmacokinetic
PD	Pharmacodynamic
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PFS6	6-month progression-free survival
PK/PD	Pharmacokinetic/Pharmacodynamic
PR	Partial Response
PTV	Planning Target Volume
QD	Once Daily
RANO	Response Assessment for Neuro-Oncology
RCC	Renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended Phase 2 Dose
RT	Radiation Treatment
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic-Oxaloacetic Transaminase (also known as AST)
SGPT	Serum Glutamic-Pyruvic Transaminase (also known as ALT)
$T_{1/2}$	Half-Life of the Terminal Disposition Rate Constant
TCR	T cell receptor
TIL	Tumor-infiltrating lymphocytes
TO	Target Occupancy
T_{reg}	Regulatory T cell
ULN	Upper Limit of Normal
WB	Whole Blood
WBC	White Blood Cell
WHO	World Health Organization
WOCBP	Women of Childbearing Potential

1 Introduction

This document is a protocol for a human research study. This study is to be conducted in accordance with US government research regulations, and applicable international standards of Good Clinical Practice, and institutional research policies and procedures.

1.1 Background

1.1.1 Study Disease

About 15,000 new glioblastomas (GBM, WHO grade IV) are diagnosed each year in the US,[1] and nearly all are fatal. Even with the relatively recent US FDA approvals of therapies for the treatment of either newly diagnosed (temozolomide in 2005) or recurrent (bevacizumab in 2009) GBM, the prognosis of GBM has not significantly changed in over 50 years. In recent multi-institutional randomized trials of newly diagnosed GBM, median survival times have been 15-16 months.[2-6] Standard therapy for newly diagnosed GBM, which consists of 6 weeks of fractionated radiation to 60 Gy with concurrent and adjuvant temozolomide, results in a modest improvement in survival of patients with *MGMT* promoter methylated GBM tumors.[3,7] However, patients with unmethylated *MGMT* GBM, which constitutes approximately half of all GBM, derive little, if any, benefit from temozolomide.[7] Patients with *MGMT* unmethylated GBM have a particularly dismal median survival of 12 months and only 6% survive 2 years.[7] Therefore, novel treatment paradigms are desperately needed for this particularly poor prognosis molecular subset of GBM patients.

1.1.2 Rationale for Immune Checkpoint Inhibitor in Glioblastoma

Cancer immunotherapy agents have emerged as highly effective for the treatment of several cancer types, including melanoma, head and neck squamous cell, prostate cancer, renal cell carcinoma (RCC), and squamous and non-squamous non-small cell lung cancer (NSCLC). Tumors may modulate and evade the host immune response through a number of mechanisms, including down regulation of tumor-specific antigen expression and presentation, secretion of anti-inflammatory cytokines, and upregulation of inhibitory ligands. One immunotherapy strategy that has proven effective for several cancer types is enhancement of the anti-tumor immune response using agents that target T cell inhibitory checkpoint receptors such as cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1, CD279). Inhibition of the immune checkpoints using antibodies against CTLA-4, PD-1 and PD-L1 have resulted in durable regressions of several types, leading to recent US FDA approvals of several systemic agents.[8-11] T cell checkpoint regulators such as CTLA-4 and PD-1 are cell surface molecules that, when engaged by their cognate ligands, induce signaling cascades down-regulating T cell activation and proliferation. Under normal conditions, PD-1 is expressed on the cell surface of activated peripheral CD4+ and CD8+ T-cells as well as B-cells, T_{regs} and Natural Killer (NK) cells, and down-modulate unwanted or excessive immune responses, including autoimmune reactions.[12] One proposed model by which therapeutic T cell checkpoint inhibitors derive antitumor activity is through breaking of immune tolerance to tumor cell antigens.[13,14]

CTLA-4 is a cell-surface receptor expressed by activated T cells with homology to the T-cell costimulatory molecule CD28. Although CD28 and CTLA-4 are both ligands for B7-1 (CD80) and B7-2 (CD86), they serve opposing roles in regulating T-cell activation. CD28 provides costimulatory signals required for T-cell activation, whereas CTLA-4 negatively modulates T-cell responses by raising the activation threshold for T-cell priming; thus, CTLA-4 is likely most important during priming. PD-1 binds programmed death ligand 1 (PD-L1; B7-H1 or CD274) expressed by neoplastic cells, various immune cells, mesenchymal support cells, and vascular cells. This interaction negatively regulates T-cell activation when engaged with an antigen presenting cell and/or effector function when engaged with other PD-L1-positive cells. Binding of PD-L1 to its receptors suppresses T-cell migration and proliferation and restricts cancer cell killing. Thus, PD-1 is important in regulating effector functions after CD8+ T cells are activated.[13] PD-1 and

CTLA-4 regulate distinct phases of T-cell differentiation and function, and their inhibition might need to be optimally phased for maximum efficacy. Indeed, combination therapies targeting the two checkpoints CTLA-4 and PD-1 has demonstrated improved efficacy in patients with metastatic melanoma, small cell and non-small cell lung cancer.[15-19]

The PD-1/PD-L1 receptor-ligand axis is hijacked by tumors to suppress immune control and mediate tumor immune evasion. Healthy organs express little, if any, PD-L1, whereas a number of cancers including GBM constitutively and abundantly express PD-L1 and PD-L2.[12,20] GBMs frequently have prominent diffuse/fibrillary expression of PD-L1 (61% to 88% of tumors).[21-23] In addition, GBMs also upregulate PD-L1 expression in circulating monocytes and tumor-associated macrophages (TAMs).[24] These findings suggest that the PD-1/PD-L1 pathway may be a rational target for therapy.

However, GBM tumors have a dominant immune suppressive microenvironment mediated by a variety of redundant immune mechanisms.[25,26] These include secretion of immunosuppressive factors, expression of cell surface immunosuppressive factors such as PD-L1, and presence of immune cells that mediate immunosuppression such as tumor-associated macrophages of the M2 lineage and myeloid-derived suppressor cells (MDSCs).[25,26] Moreover, membranous expression of PD-L1 on 5% of tumor cells occurs in a minority of GBM tumors (7.8%-38%),[21-23] while tumor-infiltrating lymphocytes (TILs) are generally sparse, with PD-1+ TILs reported to be identified in only a third of tumors.[21,22] These findings suggest that multimodality therapy may be required for effective immunotherapy in the majority of GBMs. Indeed, in preclinical syngeneic, orthotopic glioma mouse models, single agent immune checkpoint inhibitor efficacy has been highly variable, with several reports showing no efficacy using anti-CTLA-4 and anti-PD-1 therapy in established intracranial tumors.[27-33]

1.1.3 **Rationale for Combined Nivolumab and Ipilimumab**

Combining immunotherapeutic agents with different mechanisms of action offers the possibility of synergistic response. PD-1 and CTLA-4 are both co-inhibitory molecules, but evidence suggests that they use distinct mechanisms to limit T-cell activation. Preliminary indirect data from peripheral T-cell assessments suggests that a given T-cell checkpoint inhibitor may modulate host immune cell phenotype rendering them more susceptible to alternate checkpoint inhibitors and thereby enhancing anti-tumor activity.

Preclinical data indicate that the combination of PD-1 and CTLA-4 receptor blockade may improve antitumor activity. *In vitro* combinations of nivolumab plus ipilimumab increase IFN-γ production 2- to 7-fold over either agent alone in a mixed lymphocyte reaction. Increased antitumor activity of the combination was also observed in 3 of 5 syngeneic murine cancer models. In a murine melanoma vaccine model, blockade with either CTLA-4 or PD-1 antibodies increased the proportion of CTLA-4 and PD-1-expressing CD4/CD8 tumor infiltrating T effector cells, and dual blockade increased tumor infiltration of T effector cells and decreased intratumoral T regulatory cells, as compared to either agent alone.[34]

Nivolumab is a human monoclonal antibody (mAb) that binds PD-1 and blocks the interaction of PD-1 and its ligands PD-L1 and 2 PD-L2. Ipilimumab is a human mAb that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. For detailed information on nivolumab and ipilimumab, see Section 1.2 (Study Drugs). The combination of nivolumab and ipilimumab has been approved for unresectable melanoma in multiple countries, including the US and EU. A Phase 3 study (CA209067, n = 945) reported significantly improved PFS and ORR with the combination of nivolumab and ipilimumab versus ipilimumab alone in previously untreated melanoma. In addition, deep and durable responses were observed in previously treated, extensive stage small cell lung cancer (SCLC), with a response rate of 31.1% with the combination of nivolumab and ipilimumab.[16]

In a retrospective analysis of the melanoma phase III-067 trial, the ipilimumab plus nivolumab combination appeared to have at least additive efficacy in PD-L1 negative tumors compared to monotherapy with either agent.[18] Given that only one third of newly diagnosed GBM tumors are PD-L1 positive[21-23] based on the cutoff for positivity used in this melanoma trial, combined CTLA-4 and PD-1

inhibition may be required for efficacy in patients. Indeed, in preclinical orthotopic GL261 glioma models, combined inhibition with antibodies against CTLA-4 and PD-1 had greater preclinical efficacy against compared to vehicle control and monotherapy with either agent.[31] The combination of ipilimumab and nivolumab is currently being investigated in randomized trials of newly diagnosed (NCT02311920) and recurrent GBM (NCT02017717).

1.1.4 **Radiation Therapy to Enhance Immune Checkpoint Efficacy**

Radiation Therapy (RT) has emerged as a strategy to enhance the ability of immune therapy to augment anti-cancer immune responses.[35-37] RT and immune checkpoint therapy each activates mostly non-redundant immune stimulating mechanisms and a major contribution of radiotherapy appears to be increased T-cell receptor (TCR) diversity.[36,37] RT also induces major histocompatibility complex (MHC) class I presentation, increases antigen presentation and cytotoxic T cell (CTL) recognition of irradiated cells.[36,38,39] and enhances the diversity of the TCR repertoire of the expanded peripheral T cell clones.[37] A recent study also demonstrated that fractionated radiotherapy leads to CD8+ T-cell–dependent adaptive upregulation of tumor cell PD-L1 expression.[40]

Preclinically, short course RT substantially improves the efficacy of immune checkpoint inhibition with the combination resulting in abscopal effects and protective immunologic memory.[32,33,37,40-43] In glioma orthotopic models, combined immune checkpoint inhibitor and RT has demonstrated impressive efficacy, inducing generating robust antitumor immune responses, tumor regressions, long-term survival and generation of antitumor immunity.[32,33] Notably, one study using orthotopic, immunocompetent murine GL-261 GBM models demonstrated that the combination of RT and immune checkpoint inhibition (anti-CTLA-4 with/without 4-1BB activating antibody) was required to see survival benefit over either modality alone.[32] RT has been shown to increase tumor-infiltrating lymphocytes (TILs), including 4 fold increases in total CD4+ and 4.5 fold increases in total CD8+ cells, and also mildly increases NK cell infiltration.[38,44] Additionally, RT alone can decrease intratumoral T_{reg} levels, and RT + immune checkpoint inhibitor therapy synergize to increase cytotoxic T cell (CTL) infiltration and significantly increase the CTL to T_{reg} ratio in the tumors.[33] RT also upregulates surface MHC class I expression, thereby enhancing the presentation of normally suppressed tumor-associated antigens, while also increasing ICAM-1 expression and CXCL16 secretion.[33,38,39]

1.1.5 **Immune Suppression from Prolonged, Fractionated RT and Chemotherapy**

Although RT has been shown to enhance the efficacy of immunotherapy, the specific RT dose fractionation regimen that produces optimal immunogenicity remains unclear. Notably, it has been demonstrated that the standard RT regimen for GBM (60 Gy over 30 fractions) frequently results in immunosuppression, including lymphopenia and leukopenia despite the brain containing neither lymphatic tissue nor bone marrow.[45-49] The standard RT regimen for GBM alone, without chemotherapy, lowers total CD4 counts to < 200 cells/L in 24% of glioma patients.[47] Concurrent temozolomide exacerbates lymphopenia, lowering CD4 counts to < 200 cells/L in > 40% of patients and total lymphocyte counts (TLC) to < 500 cells/L in one third of patients.[45,46] During RT, CD4 counts drop weekly, [47] leveling off after 5 weeks, although lymphopenia can persist for at least a year. Importantly, patients with lymphopenia and CD4 counts of <200 cells/mm^3 experience tumor progression sooner and have a shorter median survival of 13.1 months compared to patients with higher CD4 counts.[46]

The cause of cranial RT-associated lymphopenia is thought to be irradiation of circulating lymphocytes as each daily fraction (2 Gy) delivers a lymphotoxic dose.[49,50] In a modeling study, 6 weeks of partial-brain RT was estimated to deliver a lymphotoxic dose to 99% of circulating blood.[50] In this model, the percentage of blood receiving lymphotoxic doses increased rapidly as the number of fractions increased, with >60% of the circulating blood receiving lymphotoxic doses by 10 fractions, suggesting lymphopenia is dependent on the total number of fractions rather than the total RT dose. Indeed, a historical study showed that in patients given the same total dose of cranial RT, lymphopenia was dependent on the number of fractions into which the total dose was divided.[49]

Additionally, a recent preclinical study demonstrated that systemic chemotherapy with nitrosourea (BCNU) was highly immunosuppressive, resulting in sustained systemic lymphodepletion even after completion of treatment.[89] Addition of systemic chemotherapy to anti-PD-1 treatment also resulted in severe lymphodepletion, severe depletion of TILs, and as a result, did not show synergistic activity against orthotopic tumors in syngeneic mouse models and was inferior to anti-PD-1 monotherapy. Importantly, systemic chemotherapy administered *prior to* anti-PD-1 treatment abrogated the survival benefit of immunotherapy as well as memory response upon tumor rechallenge.[89]

Studies in other solid tumors have demonstrated that a shorter hypofractionated RT schedule may lower the rate of severe lymphopenia and therefore may result in better outcomes, in particular, when combined with immunotherapies.[51]

1.1.6 Hypofractionated RT and Immune Checkpoint Inhibitor Combination for Newly diagnosed, *MGMT* unmethylated GBM

Preclinical data
In animal models, radiation courses longer than 5 fractions have not been reported in combination with immunotherapy.[32,33,37,40-43] In addition, the specific RT fractionation scheme utilized to stimulate an immune response has also been shown to affect tumor responses to immune checkpoint inhibitor in preclinical studies of melanoma. A three or five fraction regimen of radiation (8 Gy x 3 fractions or 6 Gy x 5 fractions in consecutive days) in one study resulted in superior responses at the primary tumor site compared to a single fraction radiation regimen (20 Gy) when combined with an anti-CTLA4 antibody.[42] In addition, in this study the three and five fraction regimens induced an "abscopal effect", which resulted in regression in non-irradiated metastases that was not observed with the single fraction regimen.

Clinical data
In newly diagnosed GBM, hypofractionated, short course RT regimens delivering therapeutic doses in 5-10 fractions have recently been shown to have similar efficacy and safety to the standard RT regimen in several phase I and II trials.[52-56] In an international randomized phase III trial conducted by the IAEA conducted an international randomized phase III trial comparing a short course RT regimen consisting of 25 Gy given over 5 consecutive daily fractions was compared to a RT regimen consisting of 40 Gy delivered in 15 fractions in elderly patients with newly diagnosed GBM and poor functional status (KPS 50-70). This study found the 25 Gy regimen to be non-inferior with no difference in quality of life.[57] This trial enrolled patients aged 65 (defined as elderly) or aged 50 with poor functional status (KPS 50-70). A recent phase I trial combined 5 fraction stereotactic radiosurgery with concurrent and adjuvant temozolomide in newly diagnosed GBM (maximum Clinical Target Volume/CTV of 150 mm^3) at total dose levels ranging from 25-45 Gy. A total of 30 patients were enrolled and only 2 protocol defined dose-limiting toxicities were observed, with the per-protocol MTD defined as 40 Gy in 5 fractions.[52] No CTCAE grade 3-5 radiation necrosis events were observed, and the median overall survival was 15.0 months (11.3 months for *MGMT* unmethylated tumors).

Hypofractionated radiotherapy schemata have been established and are now considered standard of care for elderly patients (≥65 years) diagnosed with glioblastoma. In a prospective case series, 26 patients received HFRT (45 Gy in 15 fractions) with concomitant and adjuvant temozolomide. Median overall survival was 15.6 months and comparable to the EORTC/NCIC trial.[6,58] No increased toxicities were observed.[59] Similarly, HFRT was well tolerated in a prospective series in 31 patients with newly diagnosed glioblastoma who received 45 Gy in 15 fractions.[60]

In a more recent prospective phase III study, 562 patients older than 65 years with newly diagnosed glioblastoma were randomized to receive HFRT (total dose 40 Gy in 15 fractions) alone or combined with concurrent/adjuvant temozolomide. Patients who received HFRT with temozolomide had superior median overall survival (9.3 months) compared to patients who received HFRT alone (7.6 months). More importantly, treatment was well tolerated in both treatment arms and no significant toxicities aside from the temozolomide-related hematologic side effects were seen.[61]

Furthermore, in a phase I study, HFRT (30 Gy delivered in 5 fractions) combined with pembrolizumab (an anti-PD1 antibody) and bevacizumab had an acceptable toxicity profile in patient with recurrent high-grade glioma.[62] In patients with melanoma brain metastasis, HFRT combined with immune checkpoint therapy has been reported to be well tolerated and demonstrated preliminary evidence of synergistic activity.[63]

These recent clinical studies in newly diagnosed GBM demonstrate the feasibility and safety of short-course, hypofractionated radiotherapy in newly diagnosed GBM patients who are of older age and/or have inferior performance status. Whether a more intensified shorter radiation schedule is also comparable to the SOC 60 Gy/30 fraction schedule has not been established. However, to circumvent the potential immunosuppressive side effects that have been demonstrated for patients receiving the SOC 6-week regimen, a shorter treatment course may be preferable when immunotherapy is combined with radiation. This seems to be supported by few preclinical and clinical studies.

Based on these studies, patients in this clinical trial will receive HFRT of 45 Gy in 15 fractions.

The optimal schedule for combining radiotherapy and immune checkpoint inhibitors has not been definitively established. Preclinical *in vivo* glioma and melanoma models suggest that there are no significant differences in efficacy have been observed when immune checkpoint inhibitor was given prior to, concurrent with, or 7 days after RT in glioma and melanoma *in vivo* models.[32,37] However, in one preclinical mouse study using colon cancer xenografts, significant improvements in overall survival were only observed when an anti-PD-L1 antibody was given at day 1 or 5 of a five-day fractionated radiotherapy cycle whereas anti-PD-L1 antibody given 7 days after completion of radiotherapy was completely ineffective at increasing overall survival compared with radiotherapy alone.[40] Based on these results, this study will initiate immune checkpoint inhibitor 7 days prior to the initiation of RT.

1.1.7 Neoadjuvant Immune Checkpoint Inhibitor Combination for Newly diagnosed, *MGMT* unmethylated GBM amenable to a larger tumor resection

Most recently, a phase II randomized multicenter clinical study in patients with recurrent glioblastoma suggest that treatment with the PD-1 inhibitor pembrolizumab results in significantly prolonged overall survival when given neoadjuvantly (prior to tumor re-resection) as compared to pembrolizumab adjuvantly (after tumor re-resection). This increase in overall survival was associated with upregulated T cell-activation and Interferon-γ-gene expression signatures and downregulation of cell-cycle related gene expression signatures in tumor cells. This suggests that PD-1 blockade enables tumor-specific T cell clone expansion which appears to be increased in the presence of bulk tumor. However, diversification of the T cell repertoire is dependent on the CTLA4-pathway rather than the PD-1 axis. These data therefore suggest a role for combining PD-1 with CTLA4-blockade in order to further enhance the tumor-directed T cell response.[64]

Another single-arm phase II study (NCT02550249) evaluated the feasibility, safety and immunobiological effects of the PD-1 inhibitor nivolumab administered preoperatively in 27 patients with recurrent and 3 patients with newly diagnosed glioblastoma. Similarly, this study found enhancement immune cell infiltration into the tumor and augmented TCR clonality.[65]

Based on these studies, we propose opening a second pre-surgical treatment cohort for patients with newly diagnosed MGMT un-methylated glioblastoma who received tumor biopsy or subtotal tumor resection only and are amenable to a larger tumor resection. These patients will receive the first dose of ipilimumab and nivolumab prior to surgical re-resection.

1.1.8 Summary of Background and Rationale

There is a significant rationale for combining anti-PD-L1 and anti-CTLA-4 therapy with short-course hypofractionated RT for therapy in *MGMT* unmethylated GBM patients. GBMs have a highly immunosuppressive tumor microenvironment and PD-L1 expression is observed in only a minority of tumors, therefore combined immune checkpoint inhibitor therapy may have greater probability of efficacy.

Furthermore, combination of immune checkpoint inhibitors with RT may increase the presentation of tumor neoantigens by increasing MHC class I presentation and increasing TCR diversity and potentially synergize with immune checkpoint inhibitor therapy given their non-redundant mechanisms of anti-tumor immune stimulation. Importantly, hypofractionated radiation and combination immunotherapy may be most beneficial as first line therapy in patients with newly diagnosed glioblastoma because prolonged radiation and exposure to chemotherapy may lead to significant immunosuppression and leukopenia and reduce the efficacy of checkpoint blockade.

Critically, short course, hypofractionated RT may avoid severe treatment-induced lymphopenia that may counteract therapeutic immune stimulation. Increasing fraction dose while decreasing the total fraction number (hypofractionation) could deliver a therapeutic dose while minimizing the RT dose to circulating blood. A shorter RT course could potentially avoid the sustained immunosuppression that has been reported with prolonged weekly fractionation schedules typically given for adjuvant therapy of high-grade gliomas. Therefore, our proposal to combine hypofractionated RT with combined immune checkpoint inhibitor treatment in patients with newly diagnosed, unmethylated *MGMT* GBM, a population where the additive benefit of temozolomide chemotherapy is questionable, may maximize the potential for immune checkpoint inhibitor efficacy. In addition, the particularly poor prognosis of this molecular subgroup even with maximal chemoradiation warrants alternative treatment paradigms.

Given the recent evidence that PD-1 blockade given prior to tumor resection may lead to increased efficacy and increased overall survival, we propose to open a second treatment cohort which will receive the first dose of ipilimumab/nivolumab prior to a second tumor re-resection.

1.2 Study Drugs

1.2.1 Nivolumab (BMS-936558 or MDX1106)

1.2.1.1 Background and Mechanism of Action of Nivolumab

Nivolumab is a human monoclonal antibody (immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligands 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens. Nivolumab is expressed in Chinese hamster ovary (CHO) cells and is produced using standard mammalian cell cultivation and chromatographic purification technologies. The clinical study product is a sterile solution for parenteral administration.

OPDIVO™ (nivolumab) is approved for use in multiple countries including the United States (US, Dec-2014), the European Union (EU, Jun-2015), and Japan (Jul-2014).

For detailed information on Nivolumab refer to the Investigator's Brochure.

1.2.1.2 Nonclinical Studies of Nivolumab

Nivolumab has been shown to bind specifically to the human PD-1 receptor and not to related members of the CD28 family. Nivolumab inhibits the interaction of PD-1 with its ligands, PD-L1 and PD-L2, resulting in enhanced T-cell proliferation and interferon-gamma (IFN-γ) release *in vitro*. Nivolumab binds with high affinity to activated human T-cells expressing cell surface PD-1 and to cynomolgus monkey PD-1. In a mixed lymphocyte reaction (MLR), nivolumab promoted a reproducible concentration-dependent enhancement of IFN-γ release.

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab was well tolerated at doses up to 50 mg/kg, administered weekly for 5 weeks, and at doses up to 50 mg/kg, administered

twice weekly for 27 doses. While nivolumab alone was well tolerated in cynomolgus monkeys, combination studies have highlighted the potential for enhanced toxicity when combined with other immunostimulatory agents.

In addition, an enhanced pre- and postnatal development (ePPND) study in pregnant cynomolgus monkeys with nivolumab was conducted. Administration of nivolumab at up to 50 mg/kg 2QW was well tolerated by pregnant monkeys; however, nivolumab was determined to be a selective developmental toxicant when administered from the period of organogenesis to parturition at ≥10 mg/kg (area under the concentration-time curve [AUC] from time zero to 168 hours [AUC(0 168 h)] 117,000 μg•h/mL). Specifically, increased developmental mortality (including late gestational fetal losses and extreme prematurity with associated neonatal mortality) was noted in the absence of overt maternal toxicity. There were no nivolumab-related changes in surviving infants tested throughout the 6-month postnatal period. Although the cause of these pregnancy failures was undetermined, nivolumab-related effects on pregnancy maintenance are consistent with the established role of PD-L1 in maintaining fetomaternal tolerance in mice.

1.2.1.3 **Effects of Nivolumab in Humans**

The PK, clinical activity, and safety of nivolumab have been assessed in subjects with non-small cell lung cancer (NSCLC), melanoma, clear-cell renal cell carcinoma (RCC), and classical Hodgkin Lymphoma (cHL) in addition to other tumor types. Nivolumab monotherapy is approved in multiple countries, including the US and EU, for unresectable or metastatic melanoma, previously treated metastatic NSCLC, and previously treated advanced RCC; it is also approved for the treatment of cHL in the US. In addition, nivolumab has been approved for use in combination with ipilimumab for unresectable melanoma in multiple countries, including the US and EU.

Nivolumab is being investigated both as monotherapy and in combination with chemotherapy, targeted therapies, and other immunotherapies. Additional clinical activity and safety information are available and summarized from clinical studies in squamous cell carcinoma of the head and neck [SCCHN], small cell lung cancer [SCLC], gastric cancer, urothelial cancer, hepatocellular carcinoma [HCC], colorectal cancer [CRC], and glioblastoma [nivolumab monotherapy]; SCLC, gastric cancer, NSCLC, RCC, and CRC [nivolumab combination therapy]; and Ono Pharmaceutical Co., Ltd. [ONO] studies in Japanese or Korean subjects).

*1.2.1.3.1 **Clinical Pharmacokinetics of Nivolumab***

The pharmacokinetics (PK) of nivolumab was studied in subjects over a dose range of 0.1 to 10 mg/kg administered as a single dose or as multiple doses of nivolumab every 2 or 3 weeks.

The geometric mean (% CV%) clearance (CL) was 9.5 mL/h (49.7%), geometric mean volume of distribution at steady state (Vss) was 8.0 L (30.4%), and geometric mean elimination half-life ($t_{1/2}$) was 26.7 days (101%). Steady-state concentrations of nivolumab were reached by 12 weeks when administered at 3 mg/kg Q2W, and systemic accumulation was approximately 3-fold. The exposure to nivolumab increased dose proportionally over the dose range of 0.1 to 10 mg/kg administered every 2 weeks. Additionally, nivolumab has a low potential for drug-drug interactions.

The clearance of nivolumab increased with increasing body weight. The PPK analysis suggested that the following factors had no clinically important effect on the CL of nivolumab: age (29 to 87 years), gender, race, baseline LDH, PD-L1. A PPK analysis suggested no difference in CL of nivolumab based on age, gender, race, solid tumor type, baseline tumor size, and hepatic impairment. Although ECOG status, baseline glomerular filtration rate (GFR), albumin, body weight, and mild hepatic impairment had an effect on nivolumab CL, the effect was not clinically meaningful. When nivolumab is administered in combination with ipilimumab, the CL of nivolumab was increased by 24%, whereas there was no effect on the clearance of ipilimumab.

*1.2.1.3.2 **Clinical Efficacy of Nivolumab***

Nivolumab has demonstrated durable responses exceeding 6 months as monotherapy and in combination with ipilimumab in several tumor types, including NSCLC, melanoma, RCC, cHL, SCLC, gastric cancer, urothelial cancer, HCC, and CRC. In confirmatory trials, nivolumab as monotherapy demonstrated a statistically significant improvement in OS as compared with the current standard of care in subjects with advanced or metastatic NSCLC, unresectable or metastatic melanoma, advanced RCC, or SCCHN. Nivolumab in combination with ipilimumab improved PFS and ORR over ipilimumab alone in subjects with unresectable or metastatic melanoma (see Investigator's Brochure).

*1.2.1.3.3 **Clinical Safety of Nivolumab***

The overall safety experience with nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 12,300 subjects treated to date.

For monotherapy, the safety profile is similar across tumor types. There is no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. In Phase 3 controlled studies, the safety profile of nivolumab monotherapy is acceptable in the context of the observed clinical efficacy, and manageable using established safety guidelines. Clinically relevant AEs typical of stimulation of the immune system were infrequent and manageable by delaying or stopping nivolumab treatment and timely immunosuppressive therapy or other supportive care.

In several ongoing clinical trials, the safety of nivolumab in combination with other therapeutics such as ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies is being explored. Most studies are ongoing and, as such, the safety profile of nivolumab combinations continues to evolve. The most advanced combination under development is nivolumab + ipilimumab, which is approved in subjects with unresectable or metastatic melanoma, and being studied in multiple tumor types (see Investigator's Brochure). Results to date suggest that the safety profile of nivolumab + ipilimumab combination therapy is consistent with the mechanisms of action of nivolumab and ipilimumab. The nature of the AEs is similar to that observed with either agent used as monotherapy; however, both frequency and severity of most AEs are increased with the combination.

1.2.2 Ipilimumab (BMS-734016, MDX010, MDX-CTLA4)

1.2.2.1 Background and Mechanism of Action of Ipilimumab

Ipilimumab is a fully human monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T-lymphocyte antigen 4 (CTLA-4, cluster of differentiation [CD] 152), which is expressed on a subset of activated T cells. CTLA-4, an activation-induced T-cell surface molecule, is a member of the CD28:B7 immunoglobulin superfamily that competes with CD28 for B7. CTLA-4 is a negative regulator of T-cell activity. Ipilimumab is a monoclonal antibody (mAb) that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation, including the activation and proliferation of tumor infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell (T_{reg}) function, which may contribute to a general increase in T-cell responsiveness, including the anti-tumor response.

Yervoy™ (ipilimumab) has been approved for use in over 47 countries including the United States (US, Mar-2011), the European Union (EU, Jul-2011), and Australia (Jul-2011).

For detailed information on Ipilimumab refer to the Investigator's Brochure.

1.2.2.2 Nonclinical Studies of ipilimumab

Ipilimumab has specificity and a high affinity for human CTLA-4. The calculated dissociation constant value from an average of several studies was 5.25 nM. Binding of ipilimumab to purified, recombinant human CTLA-4 antigen was also demonstrated by enzyme-linked immunosorbent assay with half-maximal binding at 15 ng/mL, whereas saturation was observed at approximately 0.1 μg/mL. No cross-

reactivity was observed against human CD28. Ipilimumab completely blocked binding of B7.1 and B7.2 to human CTLA-4 at concentrations higher than 6 and 1 μg/mL, respectively.

1.2.2.3 **Effects of Ipilimumab in Humans**

Bristol-Myers Squibb (BMS) has sponsored or co-sponsored an extensive clinical development program for ipilimumab, encompassing more than 19,500 subjects (total number of subjects enrolled in ipilimumab studies) in several cancer types in completed and ongoing studies, as well as a compassionate use program. The focus of the clinical program is in melanoma, prostate cancer, and lung cancer, with advanced melanoma being the most comprehensively studied indication. Ipilimumab is being investigated both as monotherapy and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapies. Phase 3 programs are ongoing in melanoma, prostate cancer, and lung cancer. In melanoma, 2 completed Phase 3 studies (MDX010-20 and CA184024) have demonstrated a clinically meaningful and statistically significant survival benefit in pretreated advanced melanoma and previously untreated advanced melanoma, respectively. Ipilimumab is in clinical development in combination with nivolumab. The combination is approved in the US for the treatment of advanced melanoma.

The safety profile of ipilimumab is generally consistent across these trials with a) the majority adverse events (AEs) being inflammatory in nature, which is consistent with the proposed mechanism of action of ipilimumab; b) the same types of such immune-mediated events in the gastrointestinal (GI) tract, skin, liver, and endocrine system being reported; and c) most of these events being manageable with immune suppressive therapies.

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Ipilimumab induces an immunologic response, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation (documented by biopsy in some subjects) is likely the cornerstone of the effect of ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. In some cases, inflammation may not be noted by radiological examination, and objective response is observed with the first tumor assessment in a manner seen in patients receiving other types of anti-cancer treatments. In other cases, response may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be mistaken for tumor progression on radiological evaluations. Therefore, in patients who are not experiencing rapid clinical deterioration, confirmation of progression is recommended (at the investigator's discretion) to better understand the prognosis, as well as to avoid unnecessarily initiating potentially toxic alternative therapies in subjects who might be benefiting from treatment. Immune-related response criteria were developed based on these observations to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

In metastatic diseases, stabilization is more common than response, and in some instances is associated with a slow, steady decline in tumor burden over many months, sometimes improving to partial and/or complete responses (CRs). Thus, the immune-based mechanism of action of ipilimumab results in durable disease control, sometimes with novel patterns of response, which contribute to its unique improvement in OS.

The unique immune-based mechanism of action is also reflected in the safety profile. Such immunological safety events are described as immune-related adverse events (irAEs) or immune-mediated adverse reactions (imARs). The irAEs are described as AEs of unknown etiology, which were consistent with an immune phenomenon and considered causally related to drug exposure by investigators. The irAEs primarily involve the GI tract and skin. Immune-related AEs in the liver were also observed, particularly in subjects receiving 10 mg/kg. Endocrinopathy and neuropathy were important irAEs that were observed less frequently. The early diagnosis of inflammatory events is important to initiate therapy and minimize complications. Inflammatory events are generally manageable using symptomatic or immuno-suppressive therapy as recommended through detailed diagnosis and management guidelines.

In summary, ipilimumab offers clinically meaningful and statistically significant survival benefit to patients with pretreated advanced melanoma and previously untreated advanced melanoma and evidence of clinical activity in randomized studies in other tumor types. These findings, together with evidence of a safety profile that is manageable with careful monitoring and appropriate intervention for treatment of immune-related toxicities, suggest an acceptable benefit to risk ratio.

1.2.3 **Rationale for Reduced Dose Ipilimumab**

Although the combination of Ipilimumab and nivolumab has been shown to have greater efficacy than either agent alone, there is increased incidence of treatment-related serious adverse events with the combination compared to monotherapy with either agent in previous and ongoing trials.[17-19] Recently reported and ongoing trials have therefore tested the combination of ipilimumab and nivolumab at reduced doses or reduced frequency of dosing. These studies have preliminarily reported similar efficacy to prior combination trials with reduced toxicity.[15,66]

Based on the initial data in melanoma and the activity observed with nivolumab and ipilimumab in lung cancer, the nivolumab plus ipilimumab combination has been also evaluated as first-line therapy in subjects with advanced NSCLC. In CA209012, early combination cohorts evaluated 2 dosing schedules that were studied in the CA209004 study in melanoma[15]:

- Nivolumab 1 mg/kg + ipilimumab 3 mg/kg, every 3 weeks for 4 doses, followed by nivolumab 3 mg/kg q 2 weeks (Arms G and H, n=24);
- Nivolumab 3 mg/kg + ipilimumab 1 mg/kg, every 3 weeks for 4 doses, followed by nivolumab 3 mg/kg q 2 weeks (Arms I and J, n=25)

These regimens resulted in significant toxicity, with 39% of subjects discontinuing treatment due to a treatment-related adverse event. Thus, additional combination cohorts were initiated (Arms N, O, P, Q), using lower doses of both nivolumab and ipilimumab, or the approved dose of nivolumab with less frequent dosing of ipilimumab. These new regimens were much better tolerated, and the safety data are not dissimilar to what has been observed in the nivolumab monotherapy cohort (Arm F in CA209012).

Activity was observed in all cohorts, with response rates greater than 39% in the 2 cohorts in which nivolumab was dosed at 3 mg/kg (N3). PFS and OS is also encouraging in the nivolumab 3 mg/kg cohorts. Clinical activity was observed in subjects with and without PD-L1 expressing tumors, though there was a greater magnitude of efficacy in subjects with PD-L1 expressing tumors. In subjects with PD-L1 expressing tumors (≥ 1% level), the response rate was 57% with nivolumab 3 mg/kg and ipilimumab 1 mg/kg every 6 or 12 weeks. For subjects with PD-L1 non-expressing tumors, the response rates were lower, but the subject numbers are small. For subjects with PD-L1 expressing and non-expressing tumors, the nivolumab 3 mg/kg every 2 weeks plus ipilimumab 1 mg/kg every 6 weeks is being further explored in a Phase 3 study in first-line NSCLC (CA209227). This dosing schedule will also be used in the experimental arm of the CA209722 study.

Based on these data, and consultation with BMS, the combination strategy for this study will be to use lower doses and less frequent dosing of ipilimumab (1 mg/kg q6 weeks) that has demonstrated acceptable safety and preliminary efficacy in ongoing combination treatment studies in lung cancer.

1.3 Research Risks & Benefits

1.3.1 **Risk of Nivolumab and Ipilimumab administration**

The safety profile of nivolumab and nivolumab plus ipilimumab is characterized by immune related toxicities, such as diarrhea, rash, pneumonitis, liver toxicity, and endocrinopathies.[17,19,67] The frequencies and intensities of these events in the combination are variable and depend on the specific doses and schedule used.[15] In the dosing schedules selected for this trial, these events have been mostly low grade and manageable with the use of corticosteroids. Nivolumab and ipilimumab combination therapy has

shown improved efficacy over either agent alone in other cancers such as melanoma.[15,17,19,67] For detailed information on the safety profiles of nivolumab and ipilimumab, refer to the Investigator's Brochures.

To minimize risk to the subject, vital signs, symptom-directed physical exams and safety labs will be performed at frequent intervals throughout the study and as indicated. In addition, subjects will be asked to report any potential adverse events and concomitant medications continually throughout the study as indicated in the Schedule of Events, and complete physical exams performed prior to the initiation of every cycle. Furthermore, investigators will be educated on expected immune-related adverse events (irAE) as defined in Section 6.2.1. Algorithms are included that describe specific irAEs and provides supportive care, dose modification and discontinuation guidelines for study drug-related adverse events (Appendix 4).

In addition, a program-wide independent Data Monitoring Committee (DMC) at BMS reviews data from the ipilimumab studies, allowing for an ongoing safety and benefit/risk assessment in subjects receiving ipilimumab. The DMC charter includes explicit stopping rules for some studies, allowing the DMC to recommend discontinuing further treatment across the ipilimumab program, if necessary.

1.3.2 **Risk with Radiation Therapy (RT)**

Radiation therapy is a standard treatment modality for GBM patients and has been established to be generally safe and well tolerated. However, the risk of radiation necrosis and other adverse events listed below may be increased with hypofractionated radiation therapy due to the increased dose given per fraction in hypofractionated prescriptions. However, in recent years, a number of small phase I and II studies and one large phase III trial have reported hypofractionated RT regimens to be well tolerated and result in similar early- and delayed-RT adverse effect profiles compared to standard radiotherapy regimens in selected newly diagnosed GBM patients.[52-57,68]

Expected acute radiation therapy adverse events include skin reaction, including dryness, redness, itching (pruritis), tanning, soreness and hair loss, fatigue, nausea, vomiting, dry mouth, altered taste, decrease in blood counts, short-term hearing impairment due to reactions in the ear canals and on the ear, serous otitis media (fluid in the middle ear) as well as temporary aggravation of brain tumor symptoms such as headaches, seizures, and weakness.

Expected early delayed radiation therapy adverse events (occurring 1-3 months after radiotherapy treatment) include fatigue, lethargy and transient worsening of existing neurological deficits.

Expected late delayed radiation therapy adverse events (occurring 3 months or more after radiation therapy treatment) include radiation necrosis (severe local damage to normal brain tissue), which may result in permanent neurocognitive deficits (difficulties with short term memory, calculations, language and more severe neurocognitive deficits) or motor dysfunction, leukoencephalopathy, permanent hearing impairment, radiation injury to the visual structures including the optic nerve and chiasm, which can result in partial or complete blindness, cataracts endocrine dysfunction and radiation-induced neoplasms. Radiation necrosis can mimic recurrent brain tumor and may require surgery for diagnosis and treatment.

To minimize risk to the subject, modern RT techniques will be used including fractionated stereotactic radiotherapy, protons, and IMRT, which allow for highly conformal treatment to a considerably more precise target volume. These technologies have the potential to significantly reduce the toxicity associated with hypofractionated radiation.[69]

1.3.3 **Potential Overlapping Toxicities with Immunotherapy and Radiation Therapy**

There is a theoretical risk that immunotherapy may potentiate the effects of central nervous system radiation necrosis given that radiation-related central nervous system necrosis is partially immune-mediated.[70-72] Thus, there is potential for increased frequency of cerebral radiation necrosis and severe

neurotoxicity from cerebral radiation necrosis. This potential for overlapping toxicity may also result in an increased risk of surgery for diagnosis and treatment of radiation necrosis.

However, a recent phase I study has demonstrated that hypofractionated re-irradiation can be safely combined with immune checkpoint therapy in recurrent high-grade glioma patients[62] and a retrospective study has reported that immune checkpoint therapy can be safely combined with various hypofractionated radiation prescriptions in melanoma brain metastasis patients.[63]

Therefore, we will conduct a Safety Lead-in (see Section 6.1.1 for details) that will include the first 6 subjects to further define the safety and tolerability of nivolumab and ipilimumab when combined with hypofractionated RT in newly diagnosed GBM subjects. Furthermore, we will conduct a special interim safety analysis for evaluation of late delayed radiation therapy adverse events due to the possibility of increased incidence of CNS toxicity from delayed radiation necrosis or radiation injury (see Section 3.5.2 for details).

1.3.4 **Other Risks of Study Participation**

Beyond the risk of study therapy exposure, subjects will not be exposed to additional risk beyond the risks associated with standard of care safety procedures for GBM therapy. The study procedures including subject visits, vital signs, phlebotomy approximately every 2 weeks, and imaging/response assessments approximately every 2 months, are equivalent to the procedures that would be performed for standard therapy for GBM. Alternative courses of management would be standard therapy with conventional fractionated radiation therapy alone, conventional fractionated radiotherapy with concurrent temozolomide chemotherapy, and no interventional therapy with palliative care only. With the study regimen, there is likely reduced risk of neurotoxicity and myelotoxicity compared to standard therapy for GBM since the study regimen consists of lower doses of radiotherapy than standard therapy regimens and does not contain a cytotoxic chemotherapy regimen.

The prognosis is so poor in newly diagnosed GBM with unmethylated *MGMT* with the standard regimen that palliative care only is considered a reasonable approach for many patients. Compared to palliative care, the risks associated with study participation outside of study therapy exposure include the risk of phlebotomy, imaging response assessments including cranial MRI, and visits with practitioners. Risks associated with phlebotomy include weakness, redness, pain, bruising, bleeding, or infection at the needle site. To minimize risk, patients will be counseled on study procedures and associated risks throughout the duration of the study.

Risk of harm from genetic testing may be a possibility should the principal investigator identify important findings in the future research and the subject opt into receiving these results. Genetic testing can generate information about a subjects' personal health risks and can cause or increase anxiety, damage family relationships, and/or compromise insurability, employability and can even lead to discrimination. Results will only be disclosed by a qualified genetic counselor under the circumstance that the PI believes they are important for the subject's health, and results will not be shared with employers, insurers, or placed in the subject's medical record, thereby greatly reducing the possibility of psychological or social risks that could arise from knowledge of this genetic information, such as risk for employability or insurability or the risk of discrimination. Additional risks to study participation include breach of confidentiality. Privacy procedures in place and good clinical practice guidelines are followed for the study to minimize risks associated with research procedures and participation.

1.3.5 **Potential benefits**

The potential benefits to subjects with study participation are improved tumor control and improved overall survival with study participation. With standard regimens, the median survival is poor for patients with GBM with unmethylated *MGMT*, and palliative care only is considered a reasonable approach for these patients.

2 Study Objectives

2.1 Research Hypothesis

This study will test the hypothesis that hypofractionated radiotherapy (45 Gy in15 fractions) will result in reduced treatment-induced severe lymphopenia compared to standard chemoradiation and enhance the efficacy of combination immune checkpoint therapy to improve the survival of newly diagnosed *MGMT*-unmethylated glioblastoma (GBM) subjects.

2.2 Overall Study Objectives

Main Study Cohort:

- The primary objective of this open-label, phase II trial will be to determine the overall survival at 1 year (1-yr OS) of adults with newly diagnosed, *MGMT* unmethylated GBM administered nivolumab, ipilimumab and hypofractionated RT.

- Secondary objectives will be to evaluate the safety and tolerability and to estimate additional measures of efficacy, as outlined in the study endpoints.

Surgical Study Cohort:

- The primary objective is to assess safety, feasibility and tolerability

- Secondary objectives will be to estimate measures of efficacy, as outlined in the study endpoints.

Both Study Cohorts:

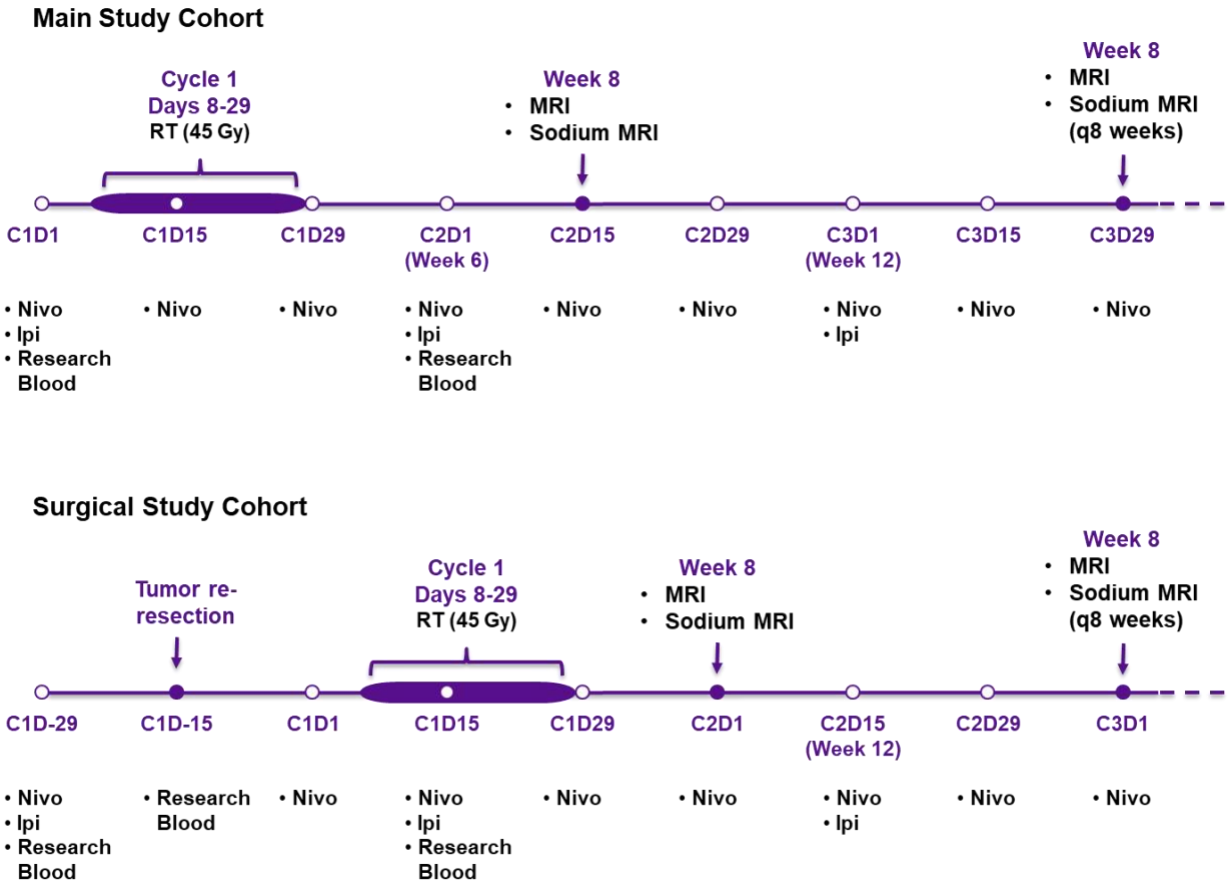
We will explore the association of 1-yr OS with tumor tissue and circulating biomarkers as described in the exploratory study endpoints to identify potential biomarkers that predict response or resistance.

3 Study Design

3.1 General Design

3.1.1 Study Schema

Timeline of Selected Important Events



3.1.2 General Study Design

This is an open label, phase II trial of nivolumab, ipilimumab and hypofractionated radiation therapy in adult patients with newly diagnosed, *MGMT* unmethylated GBM with the primary objective of determining the overall survival at 1 year.

Safety Lead In: The study will start with a Safety Lead-In consisting of the first 6 subjects (Section 6.1.1), which will be conducted to further define the safety and tolerability of nivolumab and ipilimumab when given in combination with hypofractionated radiotherapy in previously untreated, newly diagnosed GBM patients with unmethylated *MGMT* promoter. Note, special safety provisions are included for the first 3 subjects enrolled (see Section 6.1.1). After the first 6 subjects are enrolled, enrollment will stop and subjects will be observed for 40 days for dose-limiting toxicities (DLTs, see DLT definitions is Section 6.1.1.1). After all subjects in the Safety Lead-In cohort have completed the DLT observation period, the safety data will be reviewed by the Sponsor and the NYU DSMC. If 2 or more of the first 6 subjects in the Safety Lead-in experiences a DLT during the 40 day DLT observation period, the sponsor and the FDA, may redesign the dosing and schedule of the study treatment or will stop the study for unacceptable toxicity. If 1 or less of the first 6 subjects experiences a DLT at the end of the 28 day DLT observation period, the study will continue enrollment onto the phase II part of the study.

Main Study Cohort: The Phase II trial is an open-label, single-stage study at the nivolumab and ipilimumab doses and schedules determined to be safe in combination with RT in the Safety Lead-in.

The phase II trial will enroll a total of 24 subjects, including those from the safety lead-in. Monitoring of toxicity attributable to study treatment will be continually assessed during the phase II trial using a

Bayesian monitoring approach, assessing safety with the accrual of every three additional patients (see Primary Safety Endpoints, Section 3.5).

Surgical Study Cohort: We will use a single stage design to assess safety, feasibility and therapeutic efficacy of nivolumab and ipilimumab with the first dose being administered pre-operatively and continued post-operatively in conjunction with HFRT. We will enroll 16 subjects in this cohort. Monitoring of toxicity attributable to study treatment will be continually assessed (see Primary Safety Endpoints, Section 3.5).

3.1.3 General Overview Study Procedures

All Subjects: After screening procedures and registration, within 6 weeks of the first diagnostic neurosurgical procedure, all subjects will initiate study treatment with one dose of nivolumab IV (3 mg/kg) followed by one dose of ipilimumab IV (1 mg/kg).

Safety Lead-In/Main Study Cohort:

On C1D8, seven days after nivolumab and ipilimumab administration, all subjects will be administered radiotherapy (RT) to a total dose of 45 Gy, given in 15 consecutive fractions of 3 Gy each fraction.

On C1D15, subjects will receive nivolumab 3 mg/kg IV. Thereafter, nivolumab administration will continue once every 2 weeks.

On C2D1, subjects will receive ipilimumab 1 mg/kg IV. Thereafter, ipilimumab administration will continue once every 6 weeks.

Surgical Study Cohort:

Two weeks following first doses of nivolumab and ipilimumab, all subjects will undergo craniotomy and tumor re-resection (C1D-15).

On C1D1, all surgical study subjects will receive a second dose of nivolumab

On C1D8, seven days after the 2nd nivolumab dose and 3 weeks after craniotomy, all surgical study subjects will begin HFRT to a total dose of 45 Gy, given in 15 consecutive fractions of 2.6 Gy each fraction.

On C1D15, subjects will receive nivolumab 3 mg/kg and ipilimumab 1 mg/kg IV. Thereafter, nivolumab administration will continue once every 2 weeks and ipilimumab administration will continue once every 6 weeks.

All Subjects:

Nivolumab and ipilimumab administration will continue for 2 years or until progression of disease, unacceptable toxicity, death, or another protocol criterion for subject withdrawal is met, whichever comes first. One treatment cycle will be defined as 42 days (6 weeks), corresponding to 3 doses of nivolumab and one dose of ipilimumab.

Subjects will be evaluated every 8 weeks with radiographic imaging to assess response to treatment. Disease assessments on study will be made using standard Response Assessment for Neuro-Oncology (RANO) criteria for high-grade gliomas.[90] However, non-traditional Immunotherapy Response Assessment for Neuro-Oncology (iRANO) criteria (Appendix 3),[73] which is based on the immune-related response criteria and the RANO criteria for brain tumor response assessment, may be considered for patient management. The primary efficacy endpoint will be overall survival at 1 year. Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

End of treatment assessments will be performed within 7 days after last drug administration or within 7 days after decision to end treatment. In addition, site visits and safety laboratories are to be performed at 30 days (±7 days) and 90 days (±14 days) after the last study drug is given, unless the subject is unable to travel due to deteriorating medical condition, due to the potential risk for delayed immune-related toxicities. Post-treatment, all participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 100 days of the last nivolumab infusion and 90 days of the last ipilimumab infusion. Assessments may continue for ongoing reportable adverse events.

In this study, survival is a key endpoint. In addition, there is potential risk for delayed immune-related toxicities with study drug. Therefore, post study follow-up is of critical importance and is essential to preserving subject safety and the integrity of the study. Subjects who discontinue study drug must continue to be followed for collection of outcome and/or survival follow-up data until death, withdrawal of permission to record at least survival data, or subject is lost to follow-up.

Following the 90-day post-drug visit, all subjects will be contacted every 3 months (±14 days) to assess for study-treatment related toxicities, initiation of any new anti-cancer treatments, and survival status. Contact may be performed by a site visit, telephone contact, e-mail, or mail.

Expected duration of subject participation is 10 months, which is beyond the median progression-free survival achieved with standard therapy for newly diagnosed GBM with unmethylated *MGMT* (median PFS of 6 months with standard therapy).

Specific procedures to be performed during the study, as well as their required times and associated visit windows, are outlined in the Study Procedures and Schedule of Events (Appendix 1).

3.2 Primary Study Endpoint

Main Study Cohort:

- To determine the overall survival at 1 year (1-yr OS) of adults with newly diagnosed, MGMT unmethylated GBM administered nivolumab, ipilimumab and hypofractionated RT.

Surgical Study Cohort:

- The primary objective is safety, feasibility and tolerability

3.3 Secondary Study Endpoints

Main Study Cohort:

- To assess safety and tolerability
- Estimate additional measures of efficacy, including 2-year OS, median OS, median PFS, radiographic response rate, and median duration of response

Surgical Study Cohort:

- Estimate measures of efficacy, including 1-year OS, 2-year OS, median OS, median PFS, radiographic response rate, and median duration of response

3.4 Exploratory Endpoints

Both Study Cohorts:

- Association of 1-yr OS with: tumor tissue biomarkers including mutation burden, neoantigen load, immune marker expression [e.g. PD-L1, lymphocyte activation gene 3 (LAG3), indoleamine-2,3-

dioxygenase (IDO) and T-cell immunoglobulin and mucin domain 3 (TIM3)], and PD-1+ tumor infiltrating lymphocytes (TILs).

- Association of 1-yr OS with baseline and change at 6 weeks in levels of circulating biomarkers, including: regulatory T cells (T_{reg}); myeloid-derived suppressor cells (MDSC); and immune cell populations as profiled by high-parameter flow cytometry that simultaneously quantifies expression of proteins related to checkpoint inhibitors (PD1, CTLA4, TIM3, TIGIT, 41BB, CD150, LAG3, BTLA), differentiation status (CD45RA, CCR7, CD57, CD95, CD127), and other traits (CD25, IL12R, CXCR3, CXCR6).

- Effect of study regimen on total lymphocyte counts (TLC) and CD4 counts at fixed timepoints for up to 1 year compared to baseline.

- Explore whether sodium MRI, a non-invasive surrogate measure of cell death in tissue, can differentiate between treatment-related cell death/necrosis ("pseudoprogression") versus tumor progression and serve as a potential non-invasive pharmacodynamic biomarker.

3.5 Primary Safety Endpoints

3.5.1 **Phase II Safety Analysis**

For the phase II portion of the study, we consider a rate of unacceptable toxicity of 40% or greater (defined as CTCAE grade 3, grade 4, or intolerable grade 2 toxicity that is possibly, probably, or definitely related to study therapy) to be unacceptable. Intolerable grade 2 toxicity will be defined as any grade 2 toxicity that results in study discontinuation. Monitoring for toxicity will follow a Bayesian-based rule for the probability that the rate of unacceptable toxicity exceeds a maximal tolerated level of 40%.[90] We will assume a *Beta*(2,3) prior, which is prior information equivalent to two unacceptable toxicities observed in 5 treated patients. This minimally informative prior is justified as there is some clinical experience with the combination therapy. Early termination for unacceptable toxicity will be considered based on a posterior probability (in parentheses), that the unacceptable toxicity rate exceeds 30%. Toxicity will be carefully monitored in the 24 Phase II patients. Termination will be considered if unacceptable toxicity is observed in: 2 of 3 patients (0.71), 4 of 6 patients (0.83), 5 of 9 patients (0.77), 7 of 12 patients (0.86), 8 of 15 patients (0.81), 9 of 18 patients (0.77), 11 of 21 patients (0.85), or 12 of 24 patients (0.81).

3.5.2 **Special Interim Safety Analysis**

A special interim analysis for evaluation of late delayed radiation therapy adverse events (occurring 3 months or more after radiation therapy treatment) will be conducted due to a possible increased incidence of CNS toxicity from delayed radiation necrosis or radiation injury occurring during the first 6 months post RT. After the first 12 subjects treated, including subjects in the Safety Lead-In portion of the trial, have a minimum 24 week overall survival follow-up time, an interim safety analysis for delayed treatment-induced neurotoxicity will be performed. If the incidence of CTCAE grade $\geq$3 CNS (neurologic) toxicity deemed possibly, probably, or definitely related to treatment is 33% or higher (i.e., 4 or more subjects of the first 12) in this group, the trial will be halted due to lack of safety.

4 Subject Selection

4.1 Inclusion Criteria

1. Male or female subjects aged ≥18 years.
2. Histopathological evidence of glioblastoma or gliosarcoma, WHO grade IV.
3. Tumor *MGMT* promoter DNA <u>not</u> methylated (i.e., unmethylated) by central testing.

4. There will be two study cohorts:
    - **Main Study Cohort:** Maximal tumor diameter of 6.6 cm or less. Patients with multifocal tumors are allowed if the sum of the maximal tumor diameters does not exceed 6.6 cm. Note, the maximal tumor diameter for the first 3 subjects enrolled will be 5 cm.
    - **Surgical Study Cohort:** Patients who received tumor biopsy or subtotal tumor resection and are amenable to a second tumor resection with expected post-operative tumor diameter of ≤ 6 cm (PTV of ≤ 150 cm^3)
5. Subjects must not have received any prior standard or investigational anti-tumor therapy other than surgery and must not intend to receive any standard or investigational anti-tumor therapy other than the study regimen.
6. Karnofsky performance status (Appendix 2) of ≥60.
7. Availability of a paraffin-embedded or frozen tumor-tissue block with a minimum of 1 cm^2 of tumor surface area, or 20 unstained slides from the glioblastoma tissue specimen if a tumor block cannot be submitted. . For Surgical Study Cohort: availability of pre- and post-operative tumor tissue is required.
8. Subjects must start study agent within 6 weeks from the first diagnostic surgery for glioblastoma.
9. Begin of Study Treatment:
    - An interval of at least 2 weeks for surgical resection and 1 week for stereotactic biopsy from the start of study treatment. **Main Study Cohort**: Subjects must start study agents no sooner than 2 weeks from last surgical resection and 1 week from stereotactic biopsy.
    - **Surgical Study Cohort:** Subjects must receive 1st dose of study agents 2 weeks prior to tumor re-resection and must resume study agents no sooner than 2 weeks from date of tumor re-resection.
10. A contrast-enhanced MRI must be obtained within 21 days of the first dose of study treatment.
11. Adequate hematologic, hepatic, and renal function defined by
    a. White blood cell count ≥ 2.0 x 10^9/L
    b. Absolute neutrophil count ≥ 1.5 x 10^9/L
    c. Platelet count ≥ 100 x 10^9/L
    d. Hemoglobin > 9 g/dL
    e. Serum creatinine ≤1.5 x upper limit of normal (ULN) or creatinine clearance (CrCl) ≥ 40 mL/min according to the Cockcroft-Gault formula or local institutional standard method
    f. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 3 x ULN
    g. Total bilirubin ≤ 1.5 x ULN (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL)

35. Women of child-bearing potential (WOCBP) and men able to father a child must agree to use highly effective contraception (any contraceptive method with a failure rate of less than 1% per year) while on study drug and for 23 weeks (for women) or 31 weeks (for men) after the last dose of study drug.
    a. WOCBP must have a negative serum or urine pregnancy test within 24 hours of initiation of study drug.
    b. WOCBP is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes. In addition,

women under the age of 55 must have a documented serum follicle stimulating hormone (FSH) level less than 40 mIU/mL to be defined as post-menopausal.

c. WOCBP receiving nivolumab will be instructed to adhere to contraception for a period of 23 weeks after the last dose of investigational product. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product. These durations have been calculated using the upper limit of the half-life for nivolumab (25 days) and are based on the protocol requirement that WOCBP use contraception for 5 half-lives plus 30 days and men who are sexually active with WOCBP use contraception for 5 half-lives plus 90 days.

d. Highly effective contraceptive measures include: stable use of oral contraceptives such as combined estrogen and progestogen and progestogen only hormonal contraception or other prescription pharmaceutical contraceptives for 2 or more menstrual cycles prior to screening; intrauterine device [IUD]; intrauterine hormone-releasing system (IUS); bilateral tubal ligation; vasectomy and sexual abstinence.

e. Contraception is not required for men with documented vasectomy.

f. Pregnancy testing and contraception are not required for women with documented hysterectomy or tubal ligation.

g. Women must not be breastfeeding.

36. Willing to and capable of providing written informed consent prior to any study related procedures.
37. Ability and willingness to comply with all study requirements, including scheduled visits, treatment plans, laboratory tests, and other study-related procedures.

4.2 Exclusion Criteria

1. Prior use of any standard or investigational anti-tumor therapy other than surgery
2. Planned participation in another study of an investigational agent or investigational device or planned use of any other agent or therapeutic device intended for therapy of glioma.
3. Infratentorial tumors.
4. Patients with >1 cm midline shift on postoperative, baseline brain MRI.
5. Diffuse leptomeningeal gliomatosis.
6. Known mutation of the *IDH1* or *IDH2* genes in the tumor, since glioblastomas with these mutations have different biology and are associated with improved prognosis.
    a. Documentation that no *IDH1* or *IDH2* mutations are present in the tumor by a CLIA approved laboratory is required prior to initiation of study treatment.
7. Intracranial hemorrhage grade >1 not attributable to recent neurosurgery.
8. Systemic treatment with either immunosuppressive doses of corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration.
    a. Subjects on a standard high-dose steroid taper after craniotomy or stereotactic biopsy may have received a higher dose of corticosteroids within 14 days of registration, however must be at a dose < 10 mg daily prednisone or bioequivalent per day within 5 days prior to initiation of study drug.
    b. Administration of steroids through a route known to result in a minimal systemic exposure [i.e., intranasal, intraocular, inhaled, topical, or local injection (e.g., intra-articular injection) corticosteroids (<5% of body surface area)] are permitted in the absence of active autoimmune disease.

c. Subjects requiring adrenal replacement with corticosteroids are eligible if the steroids are at doses ≤ 10 mg prednisone or bioequivalent per day in the absence of active autoimmune disease.

d. Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication) are allowed.

2. Active autoimmune disease that might deteriorate when receiving an immuno-stimulatory agent. The following conditions are <u>not exclusions</u> (subjects with the following conditions are permitted):

   a. Patients with diabetes type I, vitiligo, residual hypo- or hyperthyroidism due to autoimmune condition only requiring hormone replacement, or psoriasis not requiring systemic immunosuppressive treatment, or autoimmune conditions not expected to recur in the absence of an external trigger.

3. Prior organ transplantation, including allogeneic stem cell transplantation.
4. Known history of, or any evidence of active, non-infectious pneumonitis within the last 5 years.
5. Known severe (NCI-CTCAE v4.03 Grade 3 or 4) infusion-related allergy or acute hypersensitivity reaction attributed to any monoclonal antibody, any history of anaphylaxis, or uncontrolled asthma (that is, 3 or more features of partially controlled asthma).
6. Unable tolerate an MRI, or have a contraindication to MRI.
7. Active infection requiring systemic therapy.
8. Known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome.
9. Positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus (HCV antibody) indicating acute or chronic infection.
10. Vaccination within 4 weeks of the first dose of study drug and while on trials is prohibited except for administration of inactivated vaccines. Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.
11. Clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke (< 6 months prior to enrollment), myocardial infarction (< 6 months prior to enrollment), unstable angina, congestive heart failure (≥ New York Heart Association Classification Class II), or serious cardiac arrhythmia requiring medication.
12. Patients with another active cancer [excluding basal cell carcinoma, cervical carcinoma in situ or melanoma in situ]. Prior history of other cancer is allowed, as long as there was no active disease within the prior 2 years.
13. All other unstable, severe, or chronic medical or psychiatric conditions including colitis, inflammatory bowel disease, pneumonitis, pulmonary fibrosis, recent (within the past year) or active suicidal ideation or behavior, known alcohol or drug abuse, or laboratory abnormalities that may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study.

4.3 Prior and Concomitant Therapy

4.3.1 Concomitant Medications

Any treatment administered from the time of informed consent until 30 days after the last study treatment will be considered concomitant treatment. This includes medications and other therapies for which administration started before the study and will continue during the study, as well as any therapies started in the follow-up period to treat a study-drug–related AE.

All concomitant treatments must be recorded in the study CRF with the generic name, dose, dose unit, frequency, indication, and start/stop date, as appropriate.

4.3.2 **Permitted Concomitant Medications**

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care.

Subjects are permitted the use of topical (<5% of body surface area), ocular, intra-articular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Adrenal replacement steroid doses > 10 mg daily of prednisone or bioequivalent are permitted. A brief (less than 3 weeks) course of premedication corticosteroids for prophylaxis (eg, contrast dye allergy) or for treatment of non-autoimmune conditions (eg, delayed-type hypersensitivity reaction caused by a contact allergen) is permitted. Antihistamines and other non-steroidal anti-allergy medications are also permitted.

During the study, systemic steroids at doses > 10 mg daily of prednisone or bioequivalent is also permitted for management of symptoms related to intracranial/brain edema or central nervous system radiation necrosis, however bevacizumab is preferred over corticosteroids for these conditions. See Rules for Intracranial and Brain Edema in Section 6.2.5 for guidelines on management of symptoms related to intracranial/brain edema, bevacizumab administration, and corticosteroid use in this setting.

4.3.3 **Prohibited Medications**

The following medications are prohibited during the study (unless utilized to treat a drug related adverse event):

- Immunosuppressive agents
- Immunosuppressive doses of systemic corticosteroids (except as stated in Section 5.5.2 above)
- Any concurrent anti-neoplastic therapy (ie, chemotherapy, hormonal therapy, immunotherapy, extensive, non-palliative radiation therapy, or standard or investigational agents or devices for treatment of glioblastoma)
- Antiviral treatment for HBV or HCV
- Caution should be applied in the administration of over-the-counter medications and herbal preparations during the conduct of the study. Consultation with the Sponsor is encouraged.

If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The treating investigator should discuss any questions regarding this with the overall study PI or his designee. The final decision on any supportive therapy or vaccination rests with the treating investigator.

4.3.4 **Other Restrictions and Precautions**

It is the local imaging facility's responsibility to determine, based on subject attributes (e.g., allergy history, diabetic history and renal status), the appropriate imaging modality and contrast regimen for each subject. Imaging contraindications and contrast risks should be considered in this assessment. Subjects with renal insufficiency should be assessed as to whether or not they should receive contrast and if so, what type and dose of contrast is appropriate.

Specific to MRI, subjects with severe renal insufficiency (ie, estimated glomerular filtration rate [eGFR] < 30 mL/min/1.73m^2) are at increased risk of nephrogenic systemic fibrosis. MRI contrast should not be given to this subject population. In addition, subjects are excluded from MRI if they have metallic implants, MRI-incompatible pacemakers, etc. The ultimate decision to perform MRI in an individual

subject in this study rests with the site radiologist, the investigator, and the standard set by the local Ethics Committee.

4.4 Subject Recruitment and Screening

All efforts will be made to actively recruit and retain women and members of minority groups and their subpopulations in this study, with the objective of accruing a study population that resembles the age, gender, ethnic and racial composition of the adult U.S. population as closely as possible. The inclusion and exclusion criteria in this study should not have a negative effect on the enrollment of these populations. If any differences are observed in the outcomes of these populations, these results will be reported.

Target enrollment for this study is 24 patients over 18 months. The target accrual goal is 16 patients per year. Patients will be recruited for the study from investigator or sub-investigator clinical practices. Identification of prospective subjects will be conducted by reviewing the medical records of patients in the investigator or sub-investigator's clinical practice and the investigator and sub-investigators will use all efforts to limit its use of protected health information. For study requirements to maintain subject confidentiality and management of study information according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA), refer to Section 9.

Informed consent will take place in the investigator or sub-investigator's clinical practice per institutional guidelines when a patient is deemed potentially eligible for participation by the study investigator or sub-investigator. Patients will be asked to give medical information about themselves specific to the inclusion and exclusion criteria outlined in the protocol. Only study investigators will have access to this information. Information collected will include Participant Demographics (address, zip code, sex, race, ethnicity, initials, date of birth), Parameters for eligibility, Parameters for exclusion, and Parameters for stratifications.

The Investigator or Sub-Investigator will:

1. Obtain signed and dated informed consent from the potential subject before any study specific procedures are performed.
2. Determine patient eligibility (See Section 4)
3. Submit registration/eligibility packet to the Research Coordinator at NYUSoM Perlmutter Cancer Center CTO.
4. Receive registration confirmation from the Research Coordinator at NYUSoM Perlmutter Cancer Center CTO, including a unique study identification number assigned to the patient by the research coordinator, which will be distributed to the study team upon registration of the patient.

The informed consent process and documentation follows the established procedures of the NYUSoM Perlmutter Cancer Center Clinical Trials Office.

4.4.1 **Informed Consent**

Consent will be obtained only by a participating investigator who has completed requisite training for human subject research and has been instructed by the Principal Investigator about the research study and consent process. The participating investigator must assess the subject's capacity to provide informed consent to ensure subjects who lack capacity to provide informed consent are not enrolled. The assessment should include open-ended questions (i.e., not yes or no questions) regarding the purpose and involvement of the research. The investigator evaluating patient capacity must be an M.D. with experience in evaluating patient capacity. Investigators will review the informed consent form with patients and address any questions or concerns prior to obtaining written informed consent for participation and HIPAA authorization.

Patients who are evaluated and/or treated by physicians in the oncology program will be given a consent form describing participation in the study. Patients will be given adequate time to read the consent form. They will be given time to ask questions about the study in private exam rooms. Questions will be answered by a participating physician, nurse practitioner, or research nurse all of whom have completed requisite training for human subject research. Investigators will review the informed consent form with patients and address any questions or concerns prior to obtaining written informed consent for participation. Investigators will stress that participation in the study is completely voluntary and will not affect the care patients receive or result in any loss of benefits to which patients are otherwise entitled.

For non-English speaking patients, institutional translation services will be utilized. All procedures for consenting non-English speaking patients will be in accordance with NYU Langone Health PCC CTO guidelines and policies.

For patients who cannot read, a witness, not related to the research study will be present. The consent will be read to the patient. The patient will also be allowed to ask any questions s/he may have. The investigator will ask the patient questions to ensure s/he understands the study. If the investigator determines the subject understands the study, the patient will mark an X where his/her name would go and the witness will sign the consent form.

4.4.2 **Documentation of Consent**

The Principal Investigator or IRB-approved sub-investigator will be responsible for documentation in the medical record that consent has been obtained from all participants. A signed copy of the consent form will be given to each participant. Original consent forms and informed consent checklists will be stored in the subject's medical chart/study binder. If there are any amendments made to the protocol after initial IRB approval, all subjects will have re-consents that will also be stored in the subject's medical chart/study binder.

4.5 Registration Procedures

4.5.1 **General Guidelines**

Each patient must sign and date an informed consent form before undergoing any study specific procedure unless a procedure is being performed as part of the patient's standard of care.

Enrollment in the study requires that all inclusion and exclusion criteria have been met. Enrollment occurs upon confirmation of registration from the NYUSoM PCC Clinical Trials Office (CTO). The following materials must be submitted to the Research Coordinator for registration:

1. Complete signed and dated informed consent form
2. Complete signed and dated informed consent checklist
3. Complete signed and dated eligibility checklist
4. All supporting documentation verifying each criterion has been met

Registration will occur within 48 hours of research coordinators' receipt of all the above documents. A written confirmation of enrollment including a unique study identification number assigned by the research coordinator will be distributed to the study team upon registration.

Pretreatment evaluation will therefore be dictated by standard clinical practice. Eligible patients will be entered into the study by the study coordinator.

All patients will be required to sign a written informed consent prior to being registered on this study. Any patient not registered to the study before treatment begins will be considered ineligible and registration will be denied. Every effort will be made to answer questions raised by patients and their families or

advocates regarding the protocol and alternative therapies prior to asking a patient to sign the consent form.

Once eligibility is verified, a unique patient study number will be issued within 24 hours of receiving all required registration material. The patient will not be identified by name. This is the point, at which, the patient is considered on the study. Subjects must not start any protocol study specific procedures, unless part of standard of care prior to registration

Issues that would cause treatment delays should be discussed with the Overall Principal Investigator.

5 Study Drugs and Treatment Regimen

5.1 Description and Packaging

The study drugs must be stored in accordance with the environmental conditions (temperature, light, and humidity) as determined by the IB and/or package insert. If concerns regarding the quality or appearance of the study drug arise, the study drug should not be dispensed and contact the Sponsor immediately.

5.1.1 **Nivolumab (BMS company code: BMS-936558)**

Nivolumab (Opdivo®; Bristol-Myers Squibb) is a human IgG4 mAb that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T-cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Nivolumab is US FDA-approved as a monotherapy in BRAF wild type advanced melanoma after failing ipilimumab and in BRAF V600 mutation-positive advanced melanoma after failing a BRAF inhibitor. Nivolumab in combination with ipilimumab is US FDA-approved in BRAF wild type advanced melanoma with or without prior therapies. Further details regarding the clinical experience with nivolumab may be found in the current version of the FDA-approved Opdivo® label.

Nivolumab product is a clear to opalescent colorless to pale yellow liquid that may contain particles.

Nivolumab is presented at a concentration of 10 mg/mL in single-use glass vials containing 100 mg of nivolumab. Each vial is 10 mL per volume.

Nivolumab will be packaged in a labeled carton box with 5-10 labeled vials (open-label). Each single-use 10 mL vial contains 100 mg of nivolumab (10 mg/mL).

Nivolumab drug product must be stored at 2°C to 8°C until use, and it must not be frozen. Nivolumab drug product must be protected from light.

5.1.2 **Ipilimumab (BMS company code: BMS-734016, MDX-010)**

Ipilimumab (Yervoy®; Bristol-Myers Squibb) is a fully human mAb (IgG1κ) that binds to the CTLA-4, a negative regulator of T-cell activity. Ipilimumab binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation, including the activation and proliferation of tumor infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell function, which may contribute to a general increase in T-cell responsiveness, including the anti-tumor immune response. Combined nivolumab- (anti-PD-1) and ipilimumab-mediated inhibition results in enhanced T-cell function that is greater than the effects of either antibody alone, and results in improved anti-tumor responses in metastatic melanoma. Ipilimumab is US FDA-approved in the treatment of advanced melanoma as a monotherapy (irrespective of BRAF V600 mutation status) and in combination with nivolumab in patients with BRAF wild type disease.

Further details regarding the clinical experience with ipilimumab as SA and in combination with nivolumab may be found in the current version of the approved Yervoy® and Opdivo® labels, respectively.

Ipilimumab injection, 200 mg/40 mL (5 mg/mL), is formulated as a clear to slightly opalescent, colorless to pale yellow, sterile, nonpyrogenic, single-use, isotonic aqueous solution that may contain particles.

Ipilimumab injection, 200 mg/40 mL, is supplied in 10-cc or 50-cc Type I flint glass vials, respectively, stoppered with gray butyl stoppers and sealed with aluminum seals. The drug product is formulated at a concentration of 5 mg/mL at a pH of 7.0.

Ipilimumab will be packaged in a labeled carton box with 4 labeled vials (open-label). Each single-use 40 mL vial contains 200 mg of ipilimumab (5 mg/mL).

Ipilimumab drug product must be stored at 2°C to 8°C until use, and it must not be frozen. Ipilimumab drug product must be protected from light.

5.2 Treatment Regimen

5.2.1 **Nivolumab and Ipilimumab**

After screening procedures and registration, all subjects will be treated with study treatment, which should begin as close as possible to the date on which the participant is registered.

Nivolumab and ipilimumab will be administered in an outpatient setting as intravenous (IV) infusions; however, inpatient administration is permitted. Nivolumab and ipilimumab will be provided to patients enrolled on this study by BMS.

Nivolumab 3 mg/kg IV will be given as a 30-minute infusion every 2 weeks (±3 days of the scheduled day of drug administration).

Ipilimumab 1 mg/kg IV will be given as a 30-minute infusion every 6 weeks (±5 days of the scheduled day of drug administration).

Nivolumab and ipilimumab administration will start on Day 1 and both study drugs will continue for 2 years (defined as 96 weeks, corresponding to 16 anticipated cycles) or until disease progression, unacceptable toxicity, withdrawal of consent, the study ends, or until another protocol withdrawal criterion is met, whichever occurs first.

One treatment cycle is defined as 42 days (6 weeks), corresponding to 2 doses of nivolumab and one dose of ipilimumab.

There will be no dose escalations or reductions allowed.

Doses of nivolumab and/or ipilimumab may be interrupted, delayed, or discontinued depending on how well the subject tolerates the treatment. See section 6.2 for additional information.

5.2.2 **Radiation Therapy**

RT must be provided at the study site (NYUSoM).

On Day 8 of Cycle 1 (7 days after initiation of study drugs), all subjects will begin HFRT to a total dose of 45 Gy, given in 15 fractions of 3 Gy.

5.2.2.1 **Technique**

Intensity modulated radiotherapy (IMRT) via any method (e.g., VMAT, static field IMRT) or Stereotactic Radiotherapy (SRT) is required. Any FDA cleared external beam radiation delivery system may be used (including conventional linear accelerators, cyberknife systems, tomotherapy, proton therapy, etc.). Treatment at NYUSoM will be on Varian LINAC (TrueBeam or Edge). or Elekta Gamma Knife Icon Unit.

5.2.2.2 **Dose Specifications**

Photons: Treatment shall consist of 45 Gy delivered in 15 fractions. The treatments may extend over the weekend (e.g., 15 treatments delivered over 3-4 weeks). Target coverage and homogeneity limits and deviations are listed in **Table A1**.

Table A1: Target Coverage and Dose Limits

Dose Metric	**Per Protocol**	**Variation Acceptable**	**Deviation Unacceptable**
Volume of PTV covered by the prescription dose of 45 Gy photons	Greater than or equal to 95% of the PTV should receive greater than or equal to 45 Gy photons	Greater than or equal to 90% of the PTV receiving greater than or equal to 45 Gy photons	Less than 90% of the PTV receiving greater than or equal to 45 Gy photons. Coverage less than 90% is acceptable in areas of OAR/PTV overlap.
Minimum dose to the PTV (0.03 cc)	Greater than or equal to 40.5 Gy (90% of the prescription dose) photons	Greater than or equal to 38.25 Gy (85% of the prescription dose) photons	Less than 38.25 Gy (85% of the prescription dose) photons
Maximum dose to the PTV (0.03 cc)	Less than or equal to 48.6 Gy (108% Rx Dose) photons. For SRT, this represents a plan prescribed to the 84% or greater Isodose line)	Less than or equal to 49.5 Gy (110% Rx Dose) photons. For SRT, this represents a plan prescribed to the 80% isodose line)\	Greater than 49.5 Gy (110% Rx Dose) photons. or an isodose line less than 80% for a radiosurgical plan)

Considerations for pituitary gland contouring
Pituitary dysfunction is a known complication from brain tumor radiation therapy. Approximately 41% of adult brain tumor survivors, at an average age of 6 years after radiation exposure, have evidence of some degree of hypopituitarism, including 16% with a single hormonal deficiency, 25% with multiple deficiencies and 7% with panhypopituitarism. While patients with glioblastoma rarely survive to six years, it is still important to consider radiation-induced hypopituitarism (RIH) as a potential complication.

Typically, doses of 50-54 Gy using standard fractionation are given to low grade brain tumors, including pituitary tumors, with known (RIH) as a complication. For higher doses of radiotherapy given to suprasellar tumors such as craniopharyngiomas, RIH is more common, suggesting both a possible dose or dose-volume correlation to the side effect. Investigators have suggested that doses above approximately 40-42 Gy are associated with RIH in patients receiving standard fractionation (1.8-2.0 Gy per fraction). Single fraction radiosurgery has been used to treat secretory and non-secretory pituitary tumors and suprasellar tumors.

This protocol involves the use of hypofractionated radiotherapy with a regimen of 45 Gy delivered in 15 fractions (3 Gy per fraction). Typically, dose-fractionation comparisons are preformed using biologic

equivalent dose (BED) calculations. This makes use of an alpha-beta ratio (typically assumed to be 3 for tumors and 10 for normal tissue). For 45 Gy delivered in 15 fractions, alpha/beta of 3 yields an equivalent dose of 54 Gy and alpha/beta ratio of 10 yields and equivalent dose of 49 Gy at 2 Gy per fraction. Given that we do not know the alpha-beta of pituitary tissue with certainty and that RIH is associated with doses above 40 Gy at 2 Gy / fraction, it is reasonable to set a dose constraint for the pituitary gland.

A reasonable constraint would be to contour the pituitary gland and set the maximum point dose at 45 Gy (no more than 3 Gy per fraction) in order to lower the risk of RIH.

5.2.2.3 **Technical Factors (Equipment, Energies)**

Intensity modulated radiotherapy (IMRT) via any method (e.g., VMAT, static field IMRT) or Stereotactic Radiotherapy (SRT) is required. Any FDA cleared external beam radiation delivery system may be used (including conventional linear accelerators, cyberknife systems, tomotherapy, proton therapy, etc.). Treatment at NYUSoM will be on a Varian LINAC (TrueBeam or Edge). or Eleckta Gamma Knife Perfexion Unit.

Image-guided radiotherapy with cone-beam CT along with daily treatment kV/kV imaging will be performed daily.

Imaging for treatment planning will be obtained with the patient in the same position and immobilization device as for treatment. All patients will be positioned via a combination of rigid immobilization and daily image guidance to ensure positioning accuracy of 3 mm or better, and of a magnitude that justifies the PTV margin applied.

5.2.2.4 **Localization, Simulation, and Immobilization**

MRI fusion with CT are required for treatment planning. At least 1 of these scans must be of the patient immobilized in treatment position, and with image resolution of no worse than 1.5 mm x 1.5 mm x 3 mm. MRI sequences should include axial T1 post-contrast stereotactic image (such as MP-RAGE or FSPGR BRAVO). Additionally, a T2 sequence (e.g., FLAIR or T2, preferably stereotactic, thin slice, contiguous) is helpful to identify any non-enhancing tumor.

Immobilization must be rigid (e.g., thermoplastic masks). For daily treatment, localization will include the steps of a) immobilization with the same device used for simulation, and b) daily image guidance using at a minimum orthogonal pairs of radiographs aligned to DRRs as a computer-assisted process. Daily cone-beam CT will be used as well.

5.2.2.5 **Treatment Planning/Target Volumes**

A GTV will be defined using the simulation CT scan. Fusion of the pre- and post-operative MRI scans are fused to the CT simulation scan for target delineation.

The GTV includes the post-operative resection cavity if no residual enhancing or non-enhancing tumor is noted. The GTV also includes surrounding T2/FLAIR abnormality. A CTV represents the GTV plus a 1-2 cm margin respecting normal anatomic boundaries. Care is made to not include any enhancement or T2 signal on the post-operative scan that is due to post-surgical infarct.

A PTV expansion that is justified based on image guidance and immobilization will be applied. The PTV margin is typically 3-5 mm. Daily cone-beam CT scans will be used prior to each treatment to guide radiation therapy.

5.2.2.6 **Critical Structures**

Normal tissues to be contoured will include the brain, brainstem, optic nerves and chiasm. Planning risk volume (PRV) expansions the same size as the PTV expansion (e.g. If the PTV is 3 mm, then the PRV is 3 mm.) should be utilized for optic nerves and chiasm.

Special consideration should be given to limit the exit dose through the oral cavity and mucosa.

The treatment parameters should be modified to optimize the conformity of the prescription isodose volume to the target volume while minimizing dose to critical structures. OAR limits for newly diagnosed GBM are given in **Table A2**.

Table A2: Normal Dose Limits for Newly Diagnosed GBM (15 Fractions)

Dose Metric	Per Protocol	Deviation Unacceptable
Maximum Dose (0.03 cc) to PRV for Optic Nerves, Chiasm	Less than or equal to 40 Gy photons	Greater than 40 Gy photons
Maximum Dose (0.03 cc) to Brainstem	Less than or equal to 45 Gy photons	Greater than 45 Gy photons

Coverage of the PTV will be decreased in order to fulfill these limits.

The optic structures (chiasm and optic nerves) are kept to <267 cGy per fraction and the brainstem is allowed a maximum dose (0.03 cm) of less than or equal to the prescription dose of 45 Gy.

5.2.2.7 **Documentation Requirements**

At the completion of treatment, the following should be forwarded to the overall Principal Investigator listed below. Investigators must submit the following information:

- Daily treatment record.
- Radiotherapy summary.
- Pre-study CT or MRI (the Scan used to delineate the target volumes for planning. Submit the entire series and specify which one was used for planning).
- Isodose distributions displayed on orthogonal planes or, if not possible, on multiple transverse slices through each target.

Data should be submitted to:

Sylvia Kurz, MD, PhD
Laura and Isaac Perlmutter Cancer Center
NYU School of Medicine
240 East 38th Street, 19th Floor
New York, NY 10016

5.3 Administration of Study Drugs

5.3.1 **Nivolumab and Ipilimumab Preparation and Dosing**

Nivolumab and ipilimumab must be prepared in the site investigational pharmacy. Preparation of the diluted Nivolumab and ipilimumab for administration must be accomplished by adequate trained personnel to guarantee the sterility of the product to be injected. Only clinical site personnel who are appropriately trained on the procedure detailed in this document may perform the preparation and administration procedures. Clinical site personnel involved in these procedures must comply with all applicable regulations and standards. The administration must be performed by adequately trained personnel. Prior to dosing the subject, adhere to normal standard of care and aseptic techniques. Utilize local site procedures as appropriate.

Refer to the IB and/or package inserts for complete instructions for specific nivolumab and ipilimumab handling instructions, dose calculation, reconstitution, preparation of the infusion fluid, and administration.

Setting: Nivolumab and ipilimumab will be administered in an outpatient setting as intravenous (IV) infusions; however, inpatient administration is permitted. Nivolumab and ipilimumab should be administered in a setting that allows for immediate access to an intensive care unit or equivalent environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10 mg), epinephrine (1:1,000 dilution), allergy medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access. If an acute infusion reaction is noted, subjects should be managed according to Section 6.2.8

Nivolumab 3 mg/kg IV will be given as a 30-minute infusion every 2 weeks (±3 days of the scheduled day of drug administration).

Ipilimumab 1 mg/kg IV will be given as a 30-minute infusion every 6 weeks (±5 days of the scheduled day of drug administration).

Dosing calculations should be based on the body weight assessed. If the subject's weight on the day of dosing differs by > 10% from the weight used to calculate the prior dose, the dose must be recalculated. All doses should be rounded up or to the nearest milligram per institutional standard.

There will be no dose escalations or reductions allowed.

Subjects may be dosed with nivolumab no less than 18 days from the previous dose. Subjects may be dose with ipilimumab no less than 37 days from the previous dose.

Doses of nivolumab and/or ipilimumab may be interrupted, delayed, or discontinued depending on how well the subject tolerates the treatment. Dosing visits are not skipped, only delayed. See Section 6.2 for dose delay and discontinuation criteria.

5.3.2 Nivolumab and Ipilimumab Administration

When study drugs (nivolumab and ipilimumab) are to be administered on the same day, nivolumab is to be administered first. Nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the ipilimumab infusion.

The second infusion will always be ipilimumab and will start after the infusion line has been flushed, filters changed, and patient has been observed to ensure no infusion reaction has occurred. The time in between infusions is expected to be approximately 30 minutes but may be more or less depending on the situation.

Separate infusion bags and filters should be used when administering nivolumab and ipilimumab on the same day.

There are no premedications recommended for nivolumab or ipilimumab.

Nivolumab Injection, 100 mg/10 mL (10 mg/mL) is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding (polyethersulfone membrane) in-line filter at the protocol-specified doses. It is not to be administered as an IV push or bolus injection. Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 0.35 mg/mL. Instructions for dilution and infusion of nivolumab injection are provided in the IB and/or package insert. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent.

Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets and glass bottles.

Ipilimumab injection can be used for IV administration without dilution after transferring to a PVC, non-PVC/non-DEHP or glass container and is stable for 24 hours at 2-8°C or room temperature/ room light (RT/RL). For ipilimumab storage instructions, refer to ipilimumab IB and/or package insert.

Ipilimumab is to be administered as a 30-minute IV infusion, using a volumetric pump with a 0.2 to 1.2 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline or 5% Dextrose Injection to concentrations between 1 mg/mL and 4 mg/mL. It is not to be administered as an IV push or bolus injections. Care must be taken to assure sterility of the prepared solutions, since the drug product does not contain any antimicrobial preservatives or bacteriostatic agents. At the end of the infusion, flush the line with a sufficient quantity of normal saline or 5% dextrose solution.

Subjects should be carefully monitored for infusion reactions. Vital signs should be obtained before, after infusion and in-between ipilimuab and nivolumab infusions. If an acute infusion reaction is noted, subjects should be managed according to Section 6.2.8.

5.4 Subject Compliance Monitoring

All study drug and radiation treatments will be administered at the study site and recorded on the case report form (CRF) and captured in TrialMaster. All dosing records for each patient will be kept by the site. Patients will be administered nivolumab or ipilimumab in a clinic or hospital setting under supervision of appropriate study personnel. Radiation therapy will be administered at the study site.

Subjects who are significantly non-compliant (e.g., not complying with protocol required visits, assessments, and dosing instructions) may be withdrawn from the study by the investigator and/or sponsor. The investigator and/or sponsor have the right to discontinue a subject from study treatment or withdraw a patient from the study at any time.

All drug compliance records must be kept current and must be made available for inspection by the sponsor and regulatory agency inspectors.

5.5 Receiving, Storage, Dispensing and Return

5.5.1 **Receipt of Drug Supplies**

Bristol-Myers Squibb (BMS) will package and distribute the study drug to sites. The study drug will be shipped in transport cool containers (2^{o}C to 8^{o}C) that are monitored with temperature control devices. The study drug will be shipped to the investigational pharmacy at each site.

Upon receipt of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

5.5.2 **Storage**

See above Section 5.1 for study drug storage and handling instructions.

5.5.3 **Dispensing of Study Drug**

The NYU Investigational pharmacist or their designee pharmacist or other qualified individual will be identified at each site to prepare nivolumab and ipilimumab for administration. See Section 5.3 for nivolumab and ipilimumab administration instructions.

All drug accountability records must be kept current. The investigator must be able to account for all opened and unopened study drug. These records should contain the dates, quantity, and study medication dispensed to each patient, returned from each patient (if applicable), and disposed of at the site or returned to the sponsor or designee. All accountability records must be made available for inspection by the sponsor and regulatory agency inspectors; photocopies must be provided to the sponsor at the conclusion of the study.

5.5.4 **Return or Destruction of Study Drug**

Retention of Drug Product Vials:
All opened vials (full, partially full, and empty) may be destroyed at the site by the appropriate site personnel (e.g., pharmacist, study nurse/coordinator) following local environmental requirements and institutional policies. All destruction must be fully documented at the time of destruction on the investigational product accountability log, or equivalent document at the time of destruction.

If opened vials are not destroyed immediately following drug preparation, opened vials must be stored in sealed, clear plastic bags until destruction.

All unopened vials must be destroyed at the end of the treatment period unless BMS provides separate instructions for return.

Retention of supplies:
Normal saline solution may be disposed of according to local site procedures. Other items used in the dose preparation and administration should be disposed of according to local site procedures.

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. All unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 Overall Study Design

This is an open-label, single-arm, phase II trial of nivolumab, ipilimumab and hypofractionated radiotherapy in adults with newly diagnosed, *MGMT* unmethylated GBM.

Screening begins after the subject's initial eligibility is established and the informed consent form is signed.

If the *MGMT* test was not performed at the study site (NYUSoM), then tumor tissue (archival slides or recent tumor biopsy) must be submitted to the NYUSoM for central confirmation of *MGMT* promoter DNA methylation status. Only subjects centrally confirmed to have unmethylated *MGMT* promoter will be eligible. All subjects must initiate study treatment within 6 weeks of the first diagnostic surgery.

An initial **Safety Lead-In** of 6 subjects will be conducted to define the safety of nivolumab and ipilimumab in combination with hypofractionated RT as detailed below. Note, the patients enrolled in the Safety Lead-In cohort will subsequently be analyzed in the Main Study Cohort (see below). Also note, special safety provisions are included for the first 3 subjects enrolled (see Section 6.1.1 below). After the initial 6 subjects are enrolled in the Safety Lead-In, enrollment will stop and subjects will be observed for 40 days for **dose-limiting toxicities (DLTs)** and the safety data will be reviewed by the Sponsor and the NYU DSMC. After review of the safety data in the Safety Lead-In, the Sponsor may continue accrual onto the **phase II** portion of the study if the regimen is considered safe, redesign the dosing and schedule of the study treatment with consultation from BMS and the FDA, or stop the study for unacceptable toxicity.

After screening procedures and registration, within 6 weeks of the first diagnostic surgery for glioblastoma, all subjects will initiate study treatment with one dose of nivolumab 3 mg/kg and one dose of ipilimumab 1mg/kg.

- Patients in the **Safety Lead-In**/**Main Study Cohort** will begin hypofractionated radiotherapy (HFRT) within 1 week of first nivolumab and ipilumumab dose.
- The Surgical Study Cohort will open once the Safety Lead-In is completed. Patients in the **Surgical Study Cohort** will undergo craniotomy and tumor re-resection within 2 weeks from the first dose of nivolumab and ipilimumab. Patients will begin HFRT 3 weeks after surgery.

All subjects will receive HFRT to a total dose of 45 Gy, given in 15 consecutive fractions of 3 Gy each fraction.

All subjects will continue to receive nivolumab 3 mg/kg IV every 2 weeks and ipilimumab 1 mg/kg every 6 weeks from day of first nivolumab and ipilimumab administration.

One treatment cycle will be defined as 42 days (6 weeks), corresponding to 3 doses of nivolumab and one dose of ipilimumab.

Nivolumab and ipilimumab administration will continue for all subjects for 2 years or until disease progression, intolerable toxicity, death, or the subject meets another withdrawal criteria as per the Subject Withdrawal guidelines (Section 6.12), whichever comes first. Subjects who are discontinued from study therapy will enter the post-study follow-up phase of the study as per Section 6.10.

All subjects will be evaluated every 8 weeks with radiographic imaging to assess response to treatment. Disease assessments will be made using standard RANO criteria. However, non-traditional iRANO criteria (Appendix 3) may be considered for patient management. Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI-CTCAE version 4.03.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of trial treatments in accordance with the protocol and any applicable laws and regulations.

6.1.1 **Safety Lead-In**

The first 6 subjects enrolled onto the study will be evaluated as a Safety Lead-In cohort. After all screening, registration and baseline procedures are completed as detailed on the Schedule of Events (Appendix 1), subjects will begin the study regimen.

On Day 1 of Cycle 1, subjects will be administered one dose of nivolumab 3 mg/kg IV and one dose of ipilimumab 1 mg/kg IV.

On Day 8 of Cycle 1, subjects will be administered RT to a total dose of 45 Gy, given in 15 fractions of 3 Gy.

On Day 15 of Cycle 1, nivolumab 3 mg/kg IV will be administered, and subsequently continue once every 2 weeks.

Ipilimumab 1 mg/kg IV will continue once every 6 weeks after the first ipilimumab dose on Cycle 1 Day 1.

The **DLT evaluation period** will be defined as the **first 53 days** on study. The DLT period will start with the first infusion (**Day 1**) and will **end 4 weeks following the final day of radiation**, ie, **Days 1-53**).

The first 3 subjects:

- The first 3 patients in the safety lead-in will be enrolled in a **staggered fashion**, with at least 2 weeks from time of completion of RT to first dose of study drugs for the subsequent patient.
- For the first 3 subjects, the **maximal tumor diameter may not exceed 5 cm**.
- After the 3rd subject is enrolled, **enrollment will halt and subjects will be observed for 40 days for DLTs (see DLT definitions below)** and to assess overall safety. At the end of the DLT period for the first 3 subjects, the safety data will be reviewed by the Sponsor and the NYU DSMC. If 1 or less of the first 3 subjects experiences a DLT during the DLT observation period, the maximal tumor diameter for subsequent subjects may be increased to **6.6 cm**. If ≥ 2 of the first 3 subjects experiences a DLT during the DLT observation period, the study will halt and the sponsor, NYU DSMC, and the FDA, may redesign the dosing and schedule of the study treatment or will stop the study for unacceptable toxicity.
- Continuation of enrollment will occur once all of the first 3 subjects have completed the Day 40 safety assessments and the final safety review decisions have been made.

After the first 6 subjects are enrolled, enrollment will stop and subjects will be observed for 40 days for **DLTs**. After all subjects in the Safety Lead-In cohort have completed the DLT observation period, the safety data will be reviewed by the Sponsor and the NYU DSMC. Continuation of enrollment will occur once all of the subjects have completed the Day 40 safety assessments and the final safety review decisions, including whether the study should be discontinued or proceed to phase II, have been made.

If ≥ 2 of the first 6 subjects experiences a DLT during the 40 day DLT observation period, the sponsor, and the FDA, may redesign the dosing and schedule of the study treatment or will stop the study for unacceptable toxicity.

If ≥ 2 of the first 6 subjects are unable to complete RT due to toxicity related to the planned treatment, the study will be stopped and the overall safety will be re-assessed by the sponsor and DSMC.

If 1 or less of the first 6 subjects experiences a DLT at the end of the 40 day DLT observation period, the study will continue enrollment onto the phase II part of the study.

DLT will be determined by toxicities related to nivolumab, ipilimumab, or RT during or beginning over the first 40 days of treatment as defined below. Subjects who experience DLT will be discontinued from study therapy and will enter the post-study follow-up phase of the study. Adverse events that meet DLT criteria but occur outside the DLT window will be classified as unacceptable AEs and study treatment will be discontinued.

Subjects are evaluable for DLTs if they have received at least one dose of nivolumab and ipilimumab, have completed RT per protocol, and have completed safety assessments over the entire DLT evaluation period (days 1 through 40). Any subject who discontinues treatment or withdraws from the study prior to completing the DLT evaluation period for any reason other than the occurrence of a protocol-defined DLT or other AE leading to study treatment discontinuation will be replaced. Each replacement patient will be assigned a unique patient number. Any patient who discontinues after the DLT observation period will not be replaced.

Subjects who do not experience a DLT during the DLT period (40 days) and remain on study after the DLT period will continue nivolumab and ipilimumab administration for up to 2 years or until the subject discontinues study therapy as per protocol. All subjects who discontinue study therapy will enter the post-study follow-up phase of the study.

6.1.1.1 Definition of Dose-Limiting Toxicity (DLT) for the Safety Lead-In

The DLT period for the Safety Lead-In is considered the first 40 days of study therapy, and will include labs and other evaluations taken at C1D40 (end of the first 40 days). The frequency, time to onset, and severity of toxicities, as well as the success of standard medical management and dosing interruptions/ delays, will be analyzed to determine if a given toxicity should be considered a DLT for dose decision purposes. The final decision of whether or not the AE meets the DLT definition will be based on a careful review of all relevant data by the Sponsor and the NYU DSMC. The treating investigator may also be consulted.

A DLT is defined as any non-hematologic grade ≥3 adverse event (AE) that is at least possibly, probably or definitely related to nivolumab or ipilimumab or RT during the DLT period with the exceptions noted below.

The following will be classified as DLT:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within 7 days OR requires systemic treatment.
- Any Grade ≥ 2 drug-related pneumonitis or interstitial lung disease that does not resolve to Grade 1 within 7 days and systemic steroids (also see Pulmonary Adverse Event Management Algorithm in Appendix 4).
- Any Grade 3 drug-related bronchospasm, hypersensitivity reaction, or infusion reaction, regardless of duration.
- Any Grade 3 non-hematologic, drug-related adverse event, with the following criteria:
    - Grade 3 drug-related uveitis, pneumonitis, diarrhea, or colitis of any duration will be classified as DLT.
    - Grade ≤3 symptoms attributable to brain/intracranial edema that downgrades to Grade ≤2 within 7 days, or to Grade ≤1 or baseline within 14 days after onset will not be classified as DLT (for **rules for managing brain/intracranial edema**, see Section 6.2.5 below).
    - Any ≥ grade 2 neurological AE lasting >7 days will be considered a DLT.
- Grade ≥ 3 thrombocytopenia (<50,000 mm^3), which will be classified as DLT
- Grade ≥ 3 neutropenia (ANC < 1000 mm^3), which will be classified as DLT
- Any drug-related liver function test (LFT) abnormality that meets the following criteria will be classified as DLT (also see Hepatic Adverse Event Management Algorithm):
    - AST or ALT (> 5 x ULN) for > 2 weeks
    - AST or ALT (> 8 x ULN)
    - Total bilirubin (> 5 x ULN)
    - Concurrent AST or ALT(> 3 x ULN) and total bilirubin (> 2 x ULN)
- Grade ≥ 3 lymphopenia will not be classified as DLT as this is frequently observed at baseline in newly diagnosed GBM patients.[87]

- Isolated grade ≥ 3 electrolyte abnormalities that are asymptomatic, considered unrelated or unlikely related to study treatment, and are reversed within 48 hours with replacement will not be considered DLT. However, recurrence of the same grade ≥ 3 electrolyte abnormality during the DLT period will be considered a DLT.

While rules for adjudicating DLTs are specified above, an AE of Grade <3 (except if listed as exempt above), may also be defined as a DLT after a consultation with the overall study Principal Investigator, based on the safety profile of nivolumab and/or ipilimumab. In the absence of clinical abnormality, repeat laboratory testing will be conducted to confirm significant laboratory findings prior to designation as a DLT.

For guidance on assessing the relationship of AEs to nivolumab and ipilimumab, RT, or study procedures, see Appendix 5. The sponsor may request information to justify the causality assessment of DLTs, as needed.

Note: Se**e** Section 8.1, Adverse Event Definitions, for **expected AEs** related to nivolumab or ipilimumab.

6.1.2 **Main Study Cohort**

Following completion of the Safety Lead-In, subjects meeting eligibility criteria will be enrolled onto the phase II portion of the study (Main Study Cohort). The Phase II trial is a one-stage design phase II trial with analyses for safety (see Section 7 for Statistical Methods). A total of 24 patients are anticipated to be treated in the Main Study Cohort.

After all screening, registration and baseline procedures have been completed as detailed on the **Schedule of Events** (**Appendix 1**), subjects will begin study treatment. If the initial doses of nivolumab and ipilimumab are found to be safe, subjects in the phase II portion of the study will be treated as below:

On C1D1, subjects will be administered one dose of nivolumab 3 mg/kg IV and one dose of ipilimumab 1 mg/kg IV.

On C1D8, subjects will be administered RT to a total dose of 45 Gy, given in 15 fractions of 3 Gy on consecutive days (i.e., 5 days per week).

On C1D15, subjects will receive nivolumab 3 mg/kg. Thereafter, nivolumab 3 mg/kg IV will continue once every 2 weeks.

On C1D29, subjects will receive ipilimumab 1 mg/kg IV. Thereafter, ipilimumab 1 mg/kg IV will continue once every 6 weeks.

Nivolumab and ipilimumab administration will continue for up to 2 years or until disease progression, death, intolerable toxicity, or the subject discontinues study therapy as per any of the protocol subject withdrawal guidelines. Subjects who are discontinued from study therapy will enter the post-study follow-up phase of the study.

6.1.3 **Surgical Study Cohort**

The Surgical Study Cohort will open, once the Safety Lead-In in the Main Study Cohort is completed. We will use a single-stage design and enroll a total of 16 patients

After all screening, registration and baseline procedures have been completed as detailed on the **Schedule of Events** (**Appendix 1**), subjects will begin study treatment as below:

On C1D-29, all subjects will initiate study treatment with one dose of nivolumab IV (3 mg/kg) followed by one dose of ipilimumab IV (1 mg/kg).

On C1D-15, all subjects in this cohort will undergo craniotomy and tumor re-resection.

On C1D1, all subjects will receive nivolumab IV (3 mg/kg)

On C1D8, all subjects will begin radiotherapy (RT) to a total dose of 45 Gy, given in 15 consecutive fractions of 3 Gy each fraction.

On C1D15, subjects will receive nivolumab 3 mg/kg and ipilimumab 1 mg/kg IV. Thereafter, nivolumab administration will continue once every 2 weeks and ipilimumab administration will continue once every 6 weeks.

6.2 Dose Delay and Discontinuation Criteria

6.2.1 **Immune-Related Adverse Events (irAEs)**

Special attention must be paid to adverse events (AEs) that may be suggestive of a potential immune-mediated pathophysiology. Since inhibition of immune checkpoints stimulates the immune system, immune-mediated adverse events may occur.

Immune-related adverse events (irAEs) are AEs of immune nature (i.e., inflammatory) in the absence of a clear alternative etiology. Immune-mediated AEs are specific events (that include pneumonitis, diarrhea/colitis, hepatitis, nephritis/renal dysfunction, rash, and endocrine [adrenal insufficiency, hypothyroidism/thyroiditis, hyperthyroidism, diabetes mellitus, and hypophysitis]) for which subjects received immunosuppressive medication for treatment of the event, with the exception of endocrine events (hypothyroidism/thyroiditis, hyperthyroidism, hypophysitis, diabetes mellitus, adrenal insufficiency), which are included regardless of treatment since these events are often managed without immunosuppression.

IrAEs include events, regardless of causality, occurring within 100 days of the last dose. IrAEs are limited to subjects who received immunosuppressive medication for treatment of the event, with the exception of endocrine events (hypothyroidism/thyroiditis, hyperthyroidism, hypophysitis, diabetes mellitus, adrenal insufficiency), which are included regardless of treatment since these events are often managed without immunosuppression.

An irAE can occur shortly after the first dose or several months after the last dose of treatment. All AEs of unknown etiology associated with drug exposure should be evaluated to determine possible immune etiology. If an irAE is suspected, efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes. Subjects who develop a Grade 2 or higher irAE should be discussed immediately with the overall Principal Investigator.

Management Algorithms for Immuno-Oncology Agents

Immuno-oncology (I-O) agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Nivolumab and ipilimumab are considered immuno-oncology agents in this protocol. Early recognition and management of AEs associated with immuno-oncology agents may mitigate severe toxicity. Management Algorithms have been developed to assist investigators in assessing and managing the following groups of AEs (see **Appendix 4** and the **nivolumab and ipilimumab Investigator Brochures**):

- Gastrointestinal
- Renal
- Pulmonary

- Hepatic
- Endocrinopathy
- Skin
- Neurological (non-brain/intracranial edema related)

Notes:

- For specific **rules for managing brain/intracranial edema**, see Section 6.1.3.7 below.
- These recommendations should be seen as guidelines, and the treating physician should exercise clinical judgment based on the symptoms and condition of the individual patient
- Refer to the specific dose delay and discontinuation criteria in the following sections for management of study drug-related AEs.

6.2.2 Nivolumab and Ipilimumab Dose Modification

Once the doses of nivolumab and ipilimumab are established in the Safety Lead-In portion of the study, **no dose reductions of nivolumab and ipilimumab will be allowed**. During the Safety Lead-in portion of the study, dose reductions for an individual subject will not be allowed.

6.2.3 Nivolumab and Ipilimumab Dose Delay Criteria

If nivolumab and ipilimumab dosing is delayed, the cycle count should be interrupted.

Tumor assessments for all subjects should continue as per protocol even if dosing is delayed.

Subjects who experience an adverse event that requires a treatment delay should be monitored with appropriate laboratory testing or other clinical evaluation at least weekly until resolution.

Safety Lead-In: All subjects who experience protocol-defined **DLTs** (either during or outside the DLT observation period) during the Safety Lead-In will be required to permanently discontinue treatment with nivolumab and ipilimumab. In addition, dose delay and discontinuation guidelines are provided below.

For all subjects: Nivolumab and ipilimumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, except for fatigue, laboratory abnormalities, and brain/intracranial edema (for **rules for managing brain/intracranial edema**, see Section 6.1.3.7 below)
- Any Grade ≥ 3 skin drug-related AE
- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for Grade ≥ 2 AST, ALT, or total bilirubin level elevation
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for Grade ≥ 3 AST, ALT, or total bilirubin level elevation
- Any Grade ≥ 3 drug-related amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis does not require dose delay.
- Any other Grade ≥ 3 drug-related laboratory abnormalities with the exception of Grade 3 lymphopenia which does not require a dose delay.
- Any AE, laboratory abnormality or inter-current illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

Subjects receiving ipilimumab in combination with nivolumab that have drug-related toxicities that meet the criteria for dose delay should have both drugs (ipilimumab and nivolumab) delayed until retreatment criteria are met. Exceptions apply to the retreatment criteria after dose delay of ipilimumab

and nivolumab for Grade ≥ 3 amylase and lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and that are attributed to ipilimumab alone.

- Nivolumab may be delayed until the next planned ipilimumab dose if the next ipilimumab dose is scheduled within the next 18 days. This will permit periodic ipilimumab dosing to be synchronized with nivolumab dosing.
- Ipilimumab should be dosed at the specified interval regardless of any delays in intervening nivolumab doses. However, in order to maintain periodic synchronized dosing of ipilimumab and nivolumab, the dosing days of nivolumab and ipilimumab may be adjusted within the permitted ± 5 day window, as long as consecutive nivolumab doses are given at least 16 days apart. Ipilimumab may be delayed beyond the 5 day window if needed to synchronize with the next nivolumab dose.
- If an ipilimumab dose is delayed beyond 6 weeks from the prior ipilimumab dose, then subsequent ipilimumab doses should rescheduled to maintain the 6-week interval between consecutive ipilimumab doses.
- A dose delay of ipilimumab which results in no ipilimumab dosing for > 12 weeks requires ipilimumab discontinuation, with exceptions as noted in Section 6.2.4.2.

6.2.3.1 **Criteria to Resume Nivolumab Dosing**

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

- Subjects may resume treatment in the presence of Grade 2 fatigue.
- Subjects with Grade 2 skin toxicity can be treated if no prior experience of Grade ≥ 3 drug-related skin AE.
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters (Section 6.1.3.5) should have treatment permanently discontinued.
- For subjects 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. Subjects 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 the Sponsor.
- Subjects who received systemic corticosteroids for management of any drug-related toxicity must be off corticosteroids or have tapered down to an equivalent dose of prednisone ≤ 10 mg/day.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment after consultation with the Sponsor.
- Subjects who delay study treatment due to any Grade ≥ 3 amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis, and that is assessed by the investigator to be related to ipilimumab and not to nivolumab, may resume nivolumab when the amylase or lipase abnormality has resolved to Grade < 3. The Sponsor should be consulted prior to resuming nivolumab in such subjects
- Subjects with Grade ≤3 treatment-related brain/intracranial edema may resume nivolumab if the grade 3 AE downgrades to Grade ≤1 within 7 days after onset (for **rules for managing brain/intracranial edema**, see Section 6.2.5)

6.2.3.2 **Criteria to Resume Ipilimumab Dosing**

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

- Subjects may resume treatment in the presence of Grade 2 fatigue.
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity.
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT or total bilirubin.
- Subjects with combined Grade 2 AST/ALT and total bilirubin values meeting discontinuation parameters (Section 6.1.3.5) should have treatment permanently discontinued.
- Drug-related pulmonary toxicity, diarrhea, or colitis must have resolved to baseline before treatment is resumed.
- Subjects who received systemic corticosteroids for management of any drug-related toxicity must be off corticosteroids or have tapered down to an equivalent dose of prednisone ≤ 10 mg/day.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment after consultation with the Sponsor.
- Dose delay of ipilimumab which results in no ipilimumab dosing for > 12 weeks requires ipilimumab discontinuation, with exceptions as noted in Section 6.2.4.2.
- Ipilimumab may not be resumed sooner than 6 weeks (± 5 days) after the prior ipilimumab dose.
- Subjects with Grade ≤3 treatment-related brain/intracranial edema may resume ipilimumab if the grade 3 AE downgrades to Grade ≤2 within 7 days, or to Grade ≤1 or baseline within 14 days after onset (for **rules for managing brain/intracranial edema**, see Section 6.2.5)
- In general, subjects who meet criteria to resume ipilimumab will also have met criteria to resume nivolumab, so it should be feasible to synchronize dosing of both drugs when resuming ipilimumab. In order to facilitate this, the dosing days of nivolumab and ipilimumab may be adjusted within the permitted ± 5 day window, as long as consecutive nivolumab doses are given at least 18 days apart.
- <u>One exception to note</u> is when ipilimumab and nivolumab doses are delayed due to drug-related Grade ≥ 3 amylase or lipase abnormalities not associated with symptoms or clinical manifestations of pancreatitis. If the investigator assesses the Grade ≥ 3 amylase or lipase abnormality to be related to ipilimumab and not related to nivolumab, nivolumab may be resumed when the amylase or lipase abnormality resolves to Grade < 3 but ipilimumab may only be resumed when the amylase or lipase abnormality resolves to Grade 1 or baseline. Investigator attribution of this toxicity to the ipilimumab dosing must be clearly noted in the subject's medical chart. The Sponsor should be consulted prior to resuming nivolumab in such subjects.

6.2.4 **Treatment Discontinuation Criteria**

For all subjects, global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration' in the source data and in the case report form. Every effort should be made to document objective progression (i.e., radiographic confirmation) even after discontinuation of treatment.

The assessment for discontinuation of ipilimumab should be made <u>separately</u> from the assessment made for discontinuation of nivolumab. Although there is overlap among the discontinuation criteria, if

discontinuation criteria are met for ipilimumab but not for nivolumab, treatment with nivolumab may continue if ipilimumab is discontinued.

If a subject meets criterion for permanent discontinuation and investigator is unable to determine whether the event is related to both or one study drug, the subject should discontinue both nivolumab and ipilimumab and be taken off the treatment phase of the study.

Note: Infusion-related reactions, hypersensitivity reactions (Grades 1 to 4), should be handled according to Section 6.2.8.

Note: See Section 8.1, Adverse Event Definitions, for expected AEs related to nivolumab and ipilimumab. Brain edema is an expected AE related to RT.

Decision to discontinue is determined at the time that the treatment discontinuation criteria is met. End of treatment procedures should follow as outlined in 6.7.

6.2.4.1 **Nivolumab Dose Discontinuation**

Treatment with nivolumab should be permanently discontinued for any of the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the treatment re-initiation period OR requires systemic treatment
- Any Grade ≥ 2 drug-related pneumonitis or interstitial lung disease that does not resolve to dose delay and systemic steroids (also see Pulmonary Adverse Event Management Algorithm)
- Any Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic toxicity (other than intracranial/brain edema), hypersensitivity reaction, or infusion reaction, regardless of duration
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions:
    - Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation.
    - Grade 3 symptoms attributable to brain/intracranial edema that downgrades to Grade ≤2 within 7 days, or to Grade ≤1 or baseline within 14 days do not require discontinuation (for **rules for managing brain/intracranial edema**, see Section 6.2.5)
- Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
    - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation.
    - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation (also see Hepatic Adverse Event Management Algorithm):
        - AST or ALT > 5 x ULN for > 4 weeks
        - Any AST or ALT measurements > 20 x ULN
        - Any Total bilirubin > 3 x ULN
        - Concurrent AST or ALT(> 3 x ULN) and total bilirubin (> 1.5 x ULN)
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events, which do not require discontinuation:
    - Grade 4 neutropenia ≤ 7 days

- Grade 4 lymphopenia or leukopenia
- Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset. The Sponsor should be consulted for Grade 4 amylase or lipase abnormalities
- Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset.
- Grade 4 drug-related endocrinopathy adverse events such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose controlling agents, respectively, may not require discontinuation after discussion with and approval from the Sponsor.

- Dosing delay of nivolumab which results in treatment interruption of > 6 weeks requires treatment discontinuation with the following exceptions:
  - Dosing delays to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing delay lasting > 6 weeks from the previous dose, the Sponsor must be consulted. Tumor assessments should continue as per protocol even if dosing is delayed. Periodic study visits to assess safety and laboratory studies should also continue every 6 weeks or more frequently if clinically indicated during such dosing delays.
  - Dosing delays lasting > 6 weeks from the previous dose that occur for non-drug-related reasons may be allowed if approved by the Sponsor. Prior to re-initiating treatment in a subject with a dosing delay lasting > 6 weeks, the Sponsor must be consulted. Tumor assessments should continue as per protocol even if dosing is delayed.
  - Periodic study visits to assess safety and laboratory studies should also continue every 6 weeks or more frequently if clinically indicated during such dosing delays.
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing.

6.2.4.2 **Ipilimumab Dose Discontinuation**

Ipilimumab should be permanently discontinued if any of the following criteria are met:

- Any Grade ≥ 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within 2 weeks OR requires systemic treatment;
- Any Grade ≥ 3 bronchospasm or other hypersensitivity reaction
- Any other Grade 3 non-skin, drug-related adverse event with the following <u>exceptions</u>:
  - Grade 3 nausea and vomiting
  - Grade 3 neutropenia and thrombocytopenia
  - Symptomatic endocrinopathies which resolved (with or without hormone substitution)
  - Grade ≤3 symptoms attributable to brain/intracranial edema that downgrades to Grade ≤2 within 7 days, or to Grade ≤1 or baseline within 14 days after onset (for **rules for managing brain/intracranial edema**, see Section 6.2.5)
- Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
  - AST or ALT > 5 x ULN for > 2 weeks

  - Any AST or ALT measurements > 20 x ULN
  - Any Total bilirubin > 3 x ULN
  - Concurrent AST or ALT(> 3 x ULN) and total bilirubin (> 1.5 x ULN)

- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events, which do not require discontinuation:
  - Grade 4 neutropenia ≤ 7 days
  - Grade 4 lymphopenia or leukopenia
  - Isolated Grade 4 amylase or lipase abnormalities which are not associated with symptoms or clinical manifestations of pancreatitis. The Sponsor should be consulted for Grade 4 amylase or lipase abnormalities
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
  - Grade 4 drug-related endocrinopathy adverse events such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose controlling agents, respectively, may not require discontinuation after discussion with and approval from the Sponsor.
- Any treatment delay resulting in no ipilimumab dosing for > 12 weeks with the following exceptions:
  - Dosing delays to manage drug-related adverse events, such as prolonged steroid tapers, are allowed. Prior to re-initiating treatment in a subject with a dosing delay lasting > 12 weeks, the Sponsor must be consulted. Tumor assessments should continue as per protocol even if dosing is delayed.
  - Dosing delays lasting > 12 weeks from the previous dose that occur for non-drug related reasons may be allowed if approved by the Sponsor. Prior to re-initiating treatment in a subject with a dosing delay lasting > 12 weeks, the Sponsor must be consulted. Tumor assessments should continue as per protocol even if dosing is delayed.
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing

6.2.5 Rules for Intracranial/Brain Edema

Development of edema in the brain or central nervous system necrosis is common following fractionated brain radiation therapy and may be asymptomatic (solely a radiographic finding) or associated with mild, moderate or severe symptoms. Therefore, brain edema will be considered an **expected** event related to study therapy (RT) and will ***not*** be considered a DLT and will not require permanent study drug discontinuation unless symptoms from brain/intracranial edema meets criteria for discontinuation below.

Note, the CTCAE v4.03 term **"Edema cerebral"** only has grade 4 classification in the CTCAE v4.03. Therefore, the "Edema cerebral" CTCAE v4.03 term should be used only if the event meets **grade 4 criteria** per CTCAE v4.03 criteria (i.e., life-threatening consequences; urgent intervention indicated). **Grade 4 Edema cerebral** is defined by any of the following criteria:

- Any neurosurgical procedure is required to manage brain edema
- Requires mannitol for management of symptomatic brain edema
- Requires >8 mg per day of dexamethasone or bioequivalent for more than 7 consecutive days to manage symptoms of brain edema

- Brain edema is deemed life-threatening per judgment of the treating investigator

For study purposes, the AEs deemed attributable to brain or intracranial edema that do not meet "Edema cerebral (Grade 4)" criteria per CTCAE v4.03 should be described by the symptom (e.g. headache, nausea, focal neurological deficit) and graded for severity accordingly. In addition, the term "Nervous System Disorders - Other" may be used with a description to specify the AE.

If appropriate, the investigator should use the term "**Central nervous system necrosis**" per CTCAE 4.03 criteria if the investigator deems the radiographic findings are consistent with central nervous system radiation necrosis without symptomatic Grade 4 severity.

6.2.5.1 Criteria for Nivolumab and Ipilimumab Dose Delay and Discontinuation for symptoms attributable to brain or intracranial edema or central nervous system necrosis:

- **Grade ≤3 AEs** that are attributable **brain/intracranial edema** or **central nervous system necrosis** will not be classified as DLT (if in the Safety Lead-In cohort) and will not require permanent study drug discontinuation if:
  - The grade 3 AE downgrades to Grade ≤1 within 7 days after onset of the event with maximal supportive care, including bevacizumab (see bevacizumab guidelines below) and/or systemic corticosteroid administration (no more than 7 consecutive days of dexamethasone dosed at >8 mg per day or bioequivalent).
  - The grade 2 AE improves to grade ≤1 or baseline within 7 days after onset of the event with maximal supportive care, including bevacizumab and/or systemic corticosteroid administration (no more than 7 consecutive days of dexamethasone dosed at >8 mg per day or bioequivalent).

- **Grade 4** symptoms attributable to brain or intracranial edema or central nervous system necrosis will be considered a DLT and require immediate discontinuation of nivolumab and ipilimumab.

- **Grade 4 "Edema cerebral"** will be considered a DLT require immediate discontinuation of nivolumab and ipilimumab.

Subjects who experience Edema cerebral Grade 4 will be classified as having a DLT (if in the Safety Lead-In cohort) and will discontinue nivolumab and ipilimumab immediately and will enter the post-study follow-up phase of the study. The overall PI may request additional information on events classified as Grade 4 cerebral edema.

Note on Pseudoprogression: Tumor infiltration with immune cells may be associated with increasing enhancement and edema on brain MRI or CT (i.e. **pseudoprogression**) and may mimic therapy-related central nervous system radiation necrosis or disease progression. Due to the well-described difficulty in differentiating between immune-mediated pseudoprogression and true tumor progression using standard imaging techniques,[74] particularly in brain tumors,[73] special attention must be made to events that are suspected to be attributed to possible brain edema and central nervous system necrosis.

For guidelines on managing radiographic findings of possible treatment-related brain edema, progression or pseudoprogression, refer to Efficacy Procedures and Appendix 3, **iRANO criteria**[73]**.** A symptom or AE is initially suspected to be due to pseudoprogression or central nervous system necrosis may be later considered to be due to disease progression after review of additional imaging, biopsies and consultations with the treating investigators by the Sponsor. These events may be reviewed during the Safety Lead-In and toxicity monitoring during the phase II trial.

6.2.6 Bevacizumab for Symptoms Related to Intracranial Edema

For subjects who are suspected to have study treatment-related symptomatic intracranial or brain edema at any time during the study, nivolumab and ipilimumab dosing should be immediately interrupted. **VEGF inhibition is preferred over corticosteroids** for management of suspected intracranial or brain edema, radiation necrosis, or intracranial hypertension, due to the risk that corticosteroids may suppress immune response against the tumor. Bevacizumab was granted accelerated approved by the US FDA for use as monotherapy in progressive GBM based on two historically-controlled, single-arm or non-comparative phase II trials,[75,76], however the addition of bevacizumab to standard of care in newly diagnosed GBM or to lomustine in recurrent GBM failed to show a benefit in overall survival in multi-institutional, randomized phase III trials.[4,5,77] Therefore, the addition of bevacizumab should not alter the survival outcomes of the study regimen.

The VEGF inhibition strategy will be **bevacizumab (at a maximal dose of 10 mg/kg IV) every 2 weeks, for a minimum of 2 doses**. Nivolumab and ipilimumab may resume when symptoms related to cerebral inflammation have resolved to grade ≤1 or baseline. If treatment with nivolumab and ipilimumab is resumed, bevacizimab can be continued in conjunction with these agents as deemed appropriate but the investigator.

If VEGF inhibition does not resolve the symptoms to grade ≤ 1 or baseline, the investigator may institute systemic corticosteroids, in addition to or as a replacement for bevacizumab, at the lowest dose that is appropriate for symptom management.

If the symptoms have not improved to grade ≤ 1 or baseline by 7 days after symptom onset, the subject will come off study. If a subject requires a neurosurgical procedure or mannitol to manage grade ≥3 symptom attributable to intracranial or brain edema, or if the symptoms have not improved to grade ≤ 1 or pre-treatment baseline by 7 days after symptom onset, the AE will be considered a DLT (if in the Safety Lead-in cohort) and the subject will discontinue study therapy.

Table 2: Guidelines for Nivolumab and Ipilimumab Hold and/or Discontinuations for Symptoms Related to Intracranial/Brain Edema or Central Nervous System Necrosis

Grade	Hold Nivolumab and Ipilimumab?	Restarting Criteria	Discontinuation Criteria
1	**No**	N/A	N/A
2	**Consider withholding for persistent symptoms** Consider bevacizumab if symptoms persist[1]	Toxicity resolves to Grade 0–1 or baseline within 7 days	Toxicity does not resolve to Grade ≤ 1 or baseline within 7 days of symptom onset or requires >8 mg/day of dexamethasone or bioequivalent for >7 consecutive days to treat symptoms.
3	**Yes** Initiate bevacizumab in addition to appropriate symptomatic treatment [1]	Toxicity resolves to Grade ≤ 1 or baseline within 7 days	• Toxicity does not resolve to Grade ≤ 1 or baseline within 7 days • Requires >8 mg/day of dexamethasone or bioequivalent for >7 consecutive days to treat symptoms • Requires a neurosurgical procedure or mannitol to manage symptoms
4 [2]	Discontinue	N/A	Patient must be discontinued

Note: Per CTCAE v4.03, the AE "**Edema cerebral**" only has Grade 4 severity. The AE "Edema cerebral" should be used only if the symptom severity meets the CTCAE v4.03 criteria (i.e., "Life-threatening consequences; urgent intervention indicated"). See **Rules for Brain Edema**, **Section 6.2.5**)

[1] For subjects who develop symptoms of **Grade ≤3** attributable to **brain edema** or **central nervous system necrosis**, maximal supportive care and symptomatic treatment, including bevacizumab and/or systemic corticosteroids, should be administered as clinically appropriate. **VEGF inhibition is preferred over corticosteroids** for management of suspected intracranial or brain edema, radiation necrosis, or intracranial hypertension. The VEGF inhibition strategy will be **bevacizumab (at a maximal dose of 10 mg/kg IV) every 2 weeks, for a minimum of 2 doses**. Systemic corticosteroids should be given for the lowest duration and dose required to treat symptoms in order to avoid significant immunosuppression. If corticosteroids are required, a taper should be considered once symptoms improve to Grade 1 or less and tapered over at least 4 weeks if doses >4 mg/day of dexamethasone or bioequivalent are administered for >7 days.

[2] Subjects who experience **Edema cerebral, Grade 4** per CTCAE v4.03 criteria, must discontinue nivolumab and ipilimumab. For study criteria that qualify for Grade 4 Edema cerebral see **Rules for Brain Edema** (Section 6.2.5).

6.2.7 Hold of Radiation Therapy (RT)

Radiation therapy will be held for Grade 3 or higher radiation therapy-related, nonhematologic toxicity. Radiation therapy will resume at the discretion of the investigator at full dose when toxicity returns to Grade 1 or 0. If RT is held for greater than 14 days, the subject must come off study.

Although development of symptomatic brain edema during the actual period of radiation treatment is unlikely given the hypofractionated course of RT administered in this study, radiation therapy for any patient experiencing grade 3 or higher symptoms related to brain edema prior to completing radiation treatment should be put on hold until symptoms resolve and the case is clinically evaluated.

The assessment for discontinuation of nivolumab and ipilimumab should be made separately from the assessment made for discontinuation of RT. If subject experiences an AE that in the opinion of the investigator is SOLELY related to RT and unrelated to nivolumab and ipilimumab, then nivolumab and ipilimumab may be continued and RT may be held or discontinued. Conversely, if a subject experiences an AE that in the opinion of the investigator is SOLELY related to nivolumab and/or ipilimumab and unrelated to RT, then RT may be continued while nivolumab and/or ipilimumab may be held or discontinued.

If a subject meets criterion for permanent discontinuation and the investigator is unable to determine whether the event is related to RT, nivolumab, or ipilimumab, the subject should discontinue all study treatment and be taken off the treatment phase of the study.

6.2.8 Management of Nivolumab or Ipilimumab Infusion/Hypersensitivity Reactions

Acute infusion reactions are defined as any AE that occurs during the infusion or within 2 hours after the infusion is completed. Emergency equipment and medication for the treatment of these potential adverse effects (e.g., antihistamines, bronchodilators, IV saline, corticosteroids, acetaminophen, and/or epinephrine) must be available for immediate use.

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 reactions were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported within 24 hours to the Sponsor and reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE 4.03 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 subject 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 or ipilimumab administrations.

For Grade 2 symptoms: (moderate reaction requires 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 nivolumab or ipilimumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg; remain at bedside and monitor subject 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 subject closely. If symptoms recur, then no further nivolumab or ipilimumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF).

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 or ipilimumab infusions. If necessary, corticosteroids (up to 25 mg of SoluCortef or equivalent) may be used.

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

Immediately discontinue infusion of nivolumab or ipilimumab. Begin an IV infusion of normal saline and treat the subject as follows: Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab or ipilimumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject 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).

6.3 Screening Procedures

After providing informed consent, patients will undergo screening for eligibility to participate in the study. Screening will start within 21 days prior to initiation of study treatment (first dose of nivolumab and ipilimumab). Refer also to the **Schedule of Events** (**Appendix 1**) for complete details of study procedures.

The following procedures will be performed or obtained at **Screening** for the purpose of determining study eligibility:

- Pathology report confirming the diagnosis of glioblastoma or gliosarcoma, WHO grade IV.

- *MGMT* promoter DNA methylation status must be determined by central testing (see central *MGMT* testing details below). Only subjects with unmethylated *MGMT* promoter confirmed by central testing are eligible.
    - Frozen tumor tissue or 5 unstained slides from a region of an archival FFPE tissue specimen that contains a majority of tumor from the diagnostic surgery must be submitted to NYUSoM for *MGMT* methylation testing.
- Collection of archived tumor material for research: The subject will be asked to arrange to provide archival tumor tissue from a surgical resection that demonstrates histopathological evidence of glioblastoma (GBM, WHO grade IV) or gliosarcoma as specified in the Eligibility Criteria.
- Documentation that no *IDH1* or *IDH2* mutations are present in the tumor by a CLIA approved laboratory is required prior to initiation of study treatment.
- A contrast-enhanced MRI must be obtained within 21 days of the first dose of study treatment.
- Medical history, eligibility, and concomitant medications: The subject must be eligible by **all** of the **Subject Selection Criteria** per **Section 4**. Concomitant medications will be reviewed for allowed or prohibited medications.
- Adverse event assessment
- Chest x-ray: Chest x-ray is required at screening if not performed within 60 days prior to initiation of study treatment.
    - Note: Baseline chest x-ray is required as this may assist in subsequent clinical assessments that may occur during the study. For example, in a circumstance in which a patient presents to a provider with signs and symptoms that may be related to a pulmonary process, standard clinical practice often is to obtain a chest x-ray. In order to interpret a chest x-ray in this situation, it is clinically helpful to have a recent baseline chest x-ray on file. Hence, a baseline chest x-ray is required if not performed in the prior 60 days. In the absence of a baseline chest x-ray, the most recent chest imaging would be a CT scan of the chest, which could lead to the clinical decision to obtain another CT scan of the chest in clinical circumstances in which a chest x-ray would have been sufficient.
- 12-Lead ECG: A standard 12-lead ECG will be required only at screening. The ECG strips or reports will be retained with the source. The ECG will be reviewed by the investigator (paper or electronic tracing) and will be available for comparison with subsequent ECGs by the investigator if needed. Any ECG finding performed during the study period after screening that is judged by the investigator as a clinically significant change (worsening) compared to the baseline value will be considered an AE, recorded, and monitored. The following will be recorded on the CRF:
    - PR Interval (msec)
    - QRS Interval (msec)
    - QT Interval (msec)
    - Heart Rate (BPM; recorded from the ventricular rate).
- Physical exam and laboratories: Subjects will have the following vital signs, physical exam and laboratory procedures evaluated for eligibility if not already performed within 21 days prior to initiation of study treatment (first dose of nivolumab and ipilimumab). For complete details see the **Schedule of Events** (**Appendix 1**).
    - Complete physical exam, including neurological exam, and KPS assessment
    - Vital signs: height (height is only required at screening), weight, temperature, resting blood pressure, pulse and respiration rate.

- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and protein
- Coagulation test (required at screening only): PT/INR, PT, PTT
- Thyroid stimulating hormone (TSH), free T4
- Hepatitis B virus surface antibody, hepatitis B virus surface antigen, hepatitis B virus core antibody, and hepatitis C virus antibody
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential

6.3.1 **Central *MGMT* testing**

All subjects must have their diagnostic glioblastoma tumor specimen tested for *MGMT* promoter DNA methylation at the central lab (NYUSoM). *MGMT* methylation testing will be performed using a standard clinical *MGMT* methylation assay in a CLIA-approved laboratory at NYUSoM.

Detection of *MGMT* promoter methylation will be performed on DNA extracted from formalin-fixed, paraffin-embedded (FFPE) or snap frozen tumor specimens. The assay involves sodium bisulfite modification of DNA (EpiTect FAST DNA Bisulfite Kit, Cat# 59824) that converts unmethylated cytosine into uracil, whereas methylated cytosine (mC) in a CpG island remains unchanged. This modified DNA is then used as template for PCR. To detect DNA methylation, we will perform a standard PCR-based clinical assay, pyrosequencing (PyroMark Q24).[7,78,79]

Snap-frozen tumor tissue or 5 unstained slides from a region of an archival FFPE tissue specimen that contains a majority of tumor from the diagnostic surgery must be submitted to NYUSoM for *MGMT* testing.

Send tumor specimens for central *MGMT* testing to:

Matija Snuderl, MD
Department of Pathology, NYU School of Medicine
560 First Avenue
Tisch Hospital HW 451
New York, NY 10016
Phone: 646-501-5281
Fax: 212-263-7916
Pager: 917-205-5543
Matija.Snuderl@nyumc.org

6.4 Study Procedures Main Study Cohort

6.4.1 **Cycle 1, Day 1 (C1D1)**

One treatment cycle will be defined as 42 days, corresponding to 3 doses of nivolumab (administered every 2 weeks) and one dose of ipilimumab (administered every 6 weeks). If nivolumab and ipilimumab dosing is delayed, the cycle count should be interrupted.

After screening and registration procedures, the subject will have all of the C1D1 Safety Procedures detailed below performed prior to initiating study treatment (first dose of nivolumab and ipilimumab),

within 3 days (except MRI procedures, which can be performed within 21 days) of treatment initiation. Evaluations performed at screening that fall within 3 days of treatment initiation will not need to be repeated. The baseline labs do not need to re-meet the specific eligibility criteria, only meet criteria for dosing.

- Adverse event assessment
- Concomitant medication collection
- Complete physical exam, including neurological exam, and KPS assessment
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of scheduled ipilimumab administration), temperature, resting blood pressure, pulse and respiration rate.
    - For C1D1, the following vital signs will be collected prior to treatment, at the end of the infusions, and every 30 minutes for the first hour post-infusion (all timepoints ±10 minutes):
        - Temperature, resting blood pressure, pulse and respiration rate
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- T cell subsets (CD3, CD4, and CD8 counts)
- Thyroid stimulating hormone (TSH), free T4
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential
- Contrast-enhanced brain MRI (within 21 days of study drug initiation)
- Research MRI (sodium MRI) within 21 days of study drug initiation.
- Research blood samples. All subjects will have the below research blood samples collected. *Note*: Research blood samples will be taken at only 2 timepoints: **Cycle 1, Day 1 (pre-dose)** and **Cycle 2, Day 1** (end of week 6).
    - A total of 40 mL (four 10 mL purple top EDTA tubes) of whole blood will be drawn and immediately sent to the below sites:
        - One (1) 10 mL tube will be sent to the Center for Biospecimen Research and Development (CBRD) at NYUSoM for acquisition of whole blood, buffy coat, and plasma samples.
        - Three (3) 10 mL tubes will be sent to the Immune Monitoring Core (IMC) at NYUSoM and immediately processed for isolation of PBMCs.

Study Drugs: Nivolumab and ipilimumab will be administered in an outpatient setting as an IV infusion at the specified doses (for details see Administration of Study Drugs, Section 5.3). The day of the first nivolumab and ipilimumab infusion will be designated as Cycle 1, Day 1.
The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of scheduled study drug administration.

6.4.2 **Cycle 1, Day 8 (C1D8) - Radiation Therapy**

Seven days after the first nivolumab and ipilimumab infusion (i.e. Cycle 1, Day 8), the subject will initiate **RT**. RT must be provided at the study site (NYUSoM). Technical details for radiation therapy are provided in Section 5.2.2.

- Prior to initiating radiation therapy on C1D8, adverse event assessment, a symptom-directed physical exam and limited vital signs (temperature, resting blood pressure, pulse, and respiration rate) must be performed.

- All subjects will receive RT to a total dose of 45 Gy given as 3 Gy per fraction over 15 consecutive fractions. RT must begin 1 week after the first dose of nivolumab and ipilimumab (i.e., Day 8 of Cycle 1). HFRT should preferably be given on consecutive days (i.e., 5 days per week for approximately 3 weeks).

6.4.3 Cycle 1, Day 15 (C1D15) and Day 29 (C1D29) and all subsequent mid-cycle visits (i.e., Day 15 and 29 visits)

The subject will have the Safety Procedures detailed below performed prior to initiating study treatment. Required assessments and study drug administration windows include ±3 days of scheduled visit.

- Adverse event assessment
- Concomitant medication collection
- Symptom-directed physical exam as clinically indicated by the investigator or qualified designee at all mid-cycle visits
    - For cycle 1, day 15 (C1D15) visit only, a complete physical exam and neurological exam must be performed. For all other mid-cycle visits, only a symptom-directed physical exam is necessary.
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of scheduled study drug administration)
    - Vital signs on all treatment days subsequent to C1D1 will be assessed and documented prior to the infusions and then approximately 30 minutes after the completion of the infusions:
        - Temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein

Study Drug: **Nivolumab only** will be administered in an outpatient setting as an IV infusion at the specified doses (for details see Administration of Study Drugs, Section 5.3). The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of scheduled study drug administration.

6.4.4 Cycle 2, Day 1 (C2D1) and all subsequent Day 1 cycle visits (i.e., C3D1, C4D1)

The subject will have the C2D1 Safety Procedures detailed below performed prior to initiating study treatment. Required assessments and nivolumab administration windows include ±3 days of scheduled

visit. For administrative purposes, ipilimumab administration can be ±5 days of the scheduled administration day.

- Adverse event assessment
- Concomitant medication collection
- Complete physical exam, including neurological exam, and KPS assessment
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of ipilimumab administration)
    - Vital signs will be assessed and documented prior to the infusion and then approximately 30 minutes after the completion of the infusion:
        - Temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- T cell subsets (CD3, CD4, and CD8 counts) at C2D1, C4D1, C6D1, and C8D1 only.
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein
- Thyroid stimulating hormone (TSH), free T4
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential
- Research Blood Samples (**C2D1 only**, not required at subsequent visits): *Note*: Research blood samples will be taken at only 2 timepoints: **baseline** and **C2D1** (end of week 6). All subjects will have the below research blood samples collected.
    - NYUSoM subjects: A total of 40 mL (four 10 mL purple top EDTA tubes) of whole blood will be drawn and immediately sent to the below sites:
        - One (1) 10 mL tube will be sent to the CBRD at NYUSoM for acquisition of whole blood, buffy coat, and plasma samples.
        - Three (3) 10 mL tubes will be sent to the IMC at NYUSoM and immediately processed for isolation of PBMCs.

Study Drugs: Nivolumab and ipilimumab will be administered in an outpatient setting as an IV infusion at the specified doses defined in the Safety Lead-In or the phase II trial (for details see Administration of Study Drugs, Section 5.3). The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of study drug administration.

6.5 Study Procedures for Surgical Study Cohort

One treatment cycle will be defined as 42 days, corresponding to 3 doses of nivolumab (administered every 2 weeks) and one dose of ipilimumab (administered every 6 weeks). If nivolumab and ipilimumab dosing is delayed, the cycle count should be interrupted.

6.5.1 **Cycle 1, Day -29 (C1D-29)**

After screening and registration procedures, the subject will have all of the C1D-29 Safety Procedures detailed below performed prior to initiating study treatment (first dose of nivolumab and ipilimumab), within

3 days (except MRI procedures, which can be performed within 21 days) of treatment initiation. Evaluations performed at screening that fall within 3 days of treatment initiation will not need to be repeated. The baseline labs do not need to re-meet the specific eligibility criteria, only meet criteria for dosing.

- Adverse event assessment
- Concomitant medication collection
- Complete physical exam, including neurological exam, and KPS assessment
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of scheduled study drug administration), temperature, resting blood pressure, pulse and respiration rate.
    - For C1D-29, the following vital signs will be collected prior to treatment, at the end of the infusions, and every 30 minutes for the first hour post-infusion (all time points ±10 minutes):
        - Temperature, resting blood pressure, pulse and respiration rate
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, platelet count
- T cell subsets (CD3, CD4, and CD8 counts)
- Thyroid stimulating hormone (TSH), free T4
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential
- Contrast-enhanced brain MRI (within 21 days of study drug initiation)
- Research MRI (sodium MRI) within 21 days of study drug initiation.

  Research blood samples. All subjects will have the below research blood samples collected. *Note*: Research blood samples will be taken at 3 time points: **Cycle 1, Day -29 (pre-dose), Cycle 1, Day -15 (surgery)** and **Cycle 1, Day 15** (end of week 6).

  A total of 40 mL (four 10 mL purple top EDTA tubes) of whole blood will be drawn and immediately sent to the below sites:

    - One (1) 10 mL tube will be sent to the Center for Biospecimen Research and Development (CBRD) at NYUSoM for acquisition of whole blood, buffy coat, and plasma samples.
    - Three (3) 10 mL tubes will be sent to the Immune Monitoring Core (IMC) at NYUSoM and immediately processed for isolation of PBMCs.

Study Drugs: Nivolumab and ipilimumab will be administered in an outpatient setting as an IV infusion at the specified doses (for details see Administration of Study Drugs, Section 5.3). The day of the first nivolumab and ipilimumab infusion will be designated as Cycle 1, Day -29.
The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of scheduled study drug administration.

6.5.2 **Cycle 1, Day -15 (C1D-15)**

All patients in the Surgical study cohort will undergo **craniotomy and tumor re-resection** at least 1 week after and within **2 weeks** from first dose of ipilimumab and nivolumab. The following study procedures will be performed:

- Adverse event assessment
- Concomitant medication collection
- Symptom-directed physical exam as clinically indicated by the investigator or qualified designee at all mid-cycle visits
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of scheduled study drug administration)
    - Temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein
- During the surgery, a tumor specimen will be collected for research studies:
    - Assessment of tumor tissue biomarkers including mutation burden, neoantigen load, immune marker expression [e.g. PD-L1, lymphocyte activation gene 3 (LAG3), indoleamine-2,3dioxygenase (IDO) and T-cell immunoglobulin and mucin domain 3 (TIM3)], and PD-1+ tumor infiltrating lymphocytes (TILs).
- Research blood samples. All subjects will have the below research blood samples collected at time of surgery. *Note*: Research blood samples will be taken at 3 time points: **Cycle 1, Day -29 (pre-dose), Cycle 1, Day -15 (surgery)** and **Cycle 1, Day 15** (end of week 6). The blood on the surgical date may be taken before or after the surgical procedure.

  A total of 40 mL (four 10 mL purple top EDTA tubes) of whole blood will be drawn and immediately sent to the below sites:
    - One (1) 10 mL tube will be sent to the Center for Biospecimen Research and Development (CBRD) at NYUSoM for acquisition of whole blood, buffy coat, and plasma samples.
    - Three (3) 10 mL tubes will be sent to the Immune Monitoring Core (IMC) at NYUSoM and immediately processed for isolation of PBMCs.

6.5.3 **Cycle 1, Day 1 (C1D1)**

Two weeks (+/-3 days) after craniotomy and tumor re-resection, the subject will return for second dose of nivolumab 3 mg/kg IV. The following study procedures will be performed:

- Adverse event assessment
- Concomitant medication collection
- Complete physical exam, including neurological exam, and KPS assessment
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of scheduled study drug administration)
    - Temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein

6.5.4 **Cycle 1, Day 8 (C1D8) – Begin of Radiation Therapy**

Within 3 weeks (+/- 3 days) from craniotomy and tumor re-resection (i.e. Cycle 1, Day 8), the subject will initiate **RT**. RT must be provided at the study site (NYUSoM). Technical details for radiation therapy are provided in Section 5.2.2.

- Prior to initiating radiation therapy on C1D8, adverse event assessment, a symptom-directed physical exam and limited vital signs (temperature, resting blood pressure, pulse, and respiration rate) must be performed.

- All subjects will receive HFRT to a total dose of 45 Gy given as 3 Gy per fraction over 15 fractions. RT must begin 3 weeks (+/- 3 days) after craniotomy and tumor re-resection (i.e., Day 8 of Cycle 1).

6.5.5 **Cycle 1, Day 15 (C1D15)**

The subject will have the Safety Procedures detailed below performed prior to initiating study treatment with nivolumab 3 mg/kg IV and ipilimumab 1 mg/kg IV. Required assessments and study drug administration windows include ±3 days of scheduled visit.

- Adverse event assessment
- Concomitant medication collection
- Symptom-directed physical exam as clinically indicated by the investigator or qualified designee at all mid-cycle visits
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of scheduled study drug administration)
    - Temperature, resting blood pressure, pulse and respiration rate.

- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.

- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, platelet count

- T cell subsets (CD3, CD4, and CD8 counts)

- Thyroid stimulating hormone (TSH), free T4

- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential

- Research blood samples. All subjects will have the below research blood samples collected. *Note*: Research blood samples will be taken at 3 time points: **Cycle 1, Day -29 (pre-dose), Cycle 1, Day -15 (surgery)** and **Cycle 1, Day 15** (end of week 6).

  A total of 40 mL (four 10 mL purple top EDTA tubes) of whole blood will be drawn and immediately sent to the below sites:

  - One (1) 10 mL tube will be sent to the Center for Biospecimen Research and Development (CBRD) at NYUSoM for acquisition of whole blood, buffy coat, and plasma samples.
  - Three (3) 10 mL tubes will be sent to the Immune Monitoring Core (IMC) at NYUSoM and immediately processed for isolation of PBMCs.

Study Drugs: Nivolumab and ipilimumab will be administered in an outpatient setting as an IV infusion at the specified doses (for details see Administration of Study Drugs, Section 5.3).
The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of scheduled study drug administration.

6.5.6 Cycle 1, Day 29 (C1D29) and all subsequent Cycle Day 29 visits (i.e., C2D29, C3D29, etc.)

The subject will have the Safety Procedures detailed below performed prior to initiating study treatment (nivolumab 3 mg/kg IV). Required assessments and study drug administration windows include ±3 days of scheduled visit.

- Adverse event assessment
- Concomitant medication collection
- Symptom-directed physical exam as clinically indicated by the investigator or qualified designee at all mid-cycle visits
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
  - Weight (can be taken within 3 days of scheduled study drug administration)
  - Temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.

- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein

Study Drug: **Nivolumab only** will be administered in an outpatient setting as an IV infusion at the specified doses (for details see Administration of Study Drugs, Section 5.3). The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of scheduled study drug administration.

6.5.7 Cycle 2, Day 1 (C2D1) and all subsequent Cycle Day 1 visits (i.e., C3D1, C4D1, etc.)

The subject will have the C2D1 Safety Procedures detailed below performed prior to initiating study treatment (nivolumab 3 mg/kg IV). Required assessments and nivolumab administration windows include ±3 days of scheduled visit.

- Adverse event assessment
- Concomitant medication collection
- Complete physical exam, including neurological exam, and KPS assessment
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of scheduled study drug administration)
        - Temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- T cell subsets (CD3, CD4, and CD8 counts) at C2D1, C4D1, C6D1, and C8D1 only.
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein
- Thyroid stimulating hormone (TSH), free T4
- Contrast-enhanced brain MRI (every 8 weeks, even cycles only, i.e. Cycle 2, Cycle 4, Cycle 6, etc.)
- Research MRI (sodium MRI) (every 8 weeks, even cycles only, i.e. Cycle 2, Cycle 4, Cycle 6, etc.)

Study Drug: **Nivolumab only** will be administered in an outpatient setting as an IV infusion at the specified doses (for details see Administration of Study Drugs, Section 5.3). The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of scheduled study drug administration.

6.5.8 Cycle 2, Day 15 (C2D15) and all subsequent Cycle Day 15 cycle visits (i.e., C3D15, C4D15, etc.)

The subject will have the C2D15 Safety Procedures detailed below performed prior to initiating study treatment. Required assessments and nivolumab administration windows include ±3 days of scheduled

visit. For administrative purposes, ipilimumab administration can be ±5 days of the scheduled administration day.

- Adverse event assessment
- Concomitant medication collection
- Symptom-directed physical exam as clinically indicated by the investigator or qualified designee at all mid-cycle visits
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of scheduled study drug administration)
    - Temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- T cell subsets (CD3, CD4, and CD8 counts) at C2D15, C4D15, C6D15, and C8D15 only.
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein
- Thyroid stimulating hormone (TSH), free T4
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential

Study Drugs: Nivolumab and ipilimumab will be administered in an outpatient setting as an IV infusion at the specified doses defined in the Safety Lead-In or the phase II trial (for details see Administration of Study Drugs, Section 5.3). The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of study drug administration.

6.5.9 Cycle 2, Day 29 (C2D29) and all subsequent Cycle Day 29 visits (i.e., C3D29, C4D29, etc.)

The subject will have the C2D1 Safety Procedures detailed below performed prior to initiating study treatment (nivolumab 3 mg/kg IV). Required assessments and nivolumab administration windows include ±3 days of scheduled visit.

- Adverse event assessment
- Concomitant medication collection
- Complete physical exam, including neurological exam, and KPS assessment
- Vital signs: When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection.
    - Weight (can be taken within 3 days of ipilimumab administration)
    - Vital signs will be assessed and documented prior to the infusion and then approximately 30 minutes after the completion of the infusion:
        - Temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST,

total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase, and lipase.

- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- T cell subsets (CD3, CD4, and CD8 counts) at C2D1, C4D1, C6D1, and C8D1 only.
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein
- Thyroid stimulating hormone (TSH), free T4
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential

Study Drug: **Nivolumab only** will be administered in an outpatient setting as an IV infusion at the specified doses (for details see Administration of Study Drugs, Section 5.3). The doses of study drug must be adjusted each cycle for changes in body weight of ≥10%. The weight used for dosing can be taken within 3 days of scheduled study drug administration.

6.6 Efficacy Procedures

6.6.1 Contrast-Enhanced Brain MRI

Efficacy assessment consisting of a **contrast-enhanced brain MRI** will be performed **every 8 weeks on study,** ±7 days. On-study imaging should follow calendar days (every 8 weeks) and should not be adjusted for delays in cycle starts. Clinicians may repeat response assessment more frequently as clinically indicated.

Tumor assessments for all subjects should continue as per protocol even if dosing is delayed.

Research MRIs (sodium MRI): All patients will have a research MRI (sodium MRI) at each efficacy assessment timepoint (i.e., at each contrast-enhanced MRI timepoint corresponding to every 8 weeks on study, ±7 days). Research MRIs will be performed on MRI equipment housed at the same NYU facility where patients obtain clinical MRIs.

6.6.2 Efficacy Assessment

Local reading (investigator assessment) will be used to determine eligibility and for participant management. Response Assessments will be performed on every brain imaging assessment performed on protocol per standard **RANO criteria**.[90] However, due to the known for patient management, non-traditional iRANO criteria may be used.[73]

- Refer to Appendix 3 for details on iRANO criteria, guidelines for assessing radiologic findings, and treatment algorithm for the assessment of progressive imaging findings in patients with neuro-oncological malignancies undergoing immunotherapy.

Evaluable for objective response. Only those participants who have measurable disease present at baseline (obtained within 14 days of Cycle 1, Day 1) scan and have received at least one dose of therapy will be considered evaluable for response. These participants will have their response classified according to the definitions stated in Appendix 3. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

6.6.3 Continuation of therapy pending confirmation of radiographic disease progression

If using iRANO criteria for patient management, iRANO recommends confirmation of disease progression on **follow-up imaging 3 months after initial radiographic progression** if there is no new

or substantially worsened neurological deficits that are not due to comorbid events or concurrent medication, and **it is 6 months or less from starting immunotherapy** (see Appendix 3 for iRANO criteria details). A decision of whether a patient should continue immunotherapy pending confirmation of radiographic disease progression should be established based on perceived benefits and risks. Continuation of immunotherapy may be considered pending follow-up imaging as long as subjects are deriving apparent clinical benefit with minimal and acceptable toxic effects.

By contrast, investigators may consider interrupting immunotherapy for subjects who need a substantial increase in corticosteroids (i.e., >4 mg of dexamethasone or equivalent per day) for evolving symptoms associated with brain edema or who have more than mild treatment-related toxic effects such as at least grade 2 irAEs. These guidelines are included to limit the likelihood of progressive immunotherapy-induced inflammatory changes leading to substantial deficits in otherwise stable or symptom-free patients. In such subjects, an interruption of immunotherapy dosing might be considered pending follow-up imaging.

Furthermore, investigators may discontinue or interrupt immunotherapy at any time if this option seems to be in the best medical interest of the subjects. As a general guidance, resumption of immunotherapy might be taken into account when systemic dexamethasone is decreased to 4 mg/day or less and the contrast-enhancing tumor burden is classified as stable disease, partial response, or complete response on a follow-up scan, or when relevant treatment-related toxic effects have resolved to grade 1 or less or pre-treatment baseline.

6.7 End of Treatment Procedures

Nivolumab and ipilimumab administration will continue for 2 years or until progression of disease, unacceptable adverse event(s), subject withdrawal of consent, death or another event that meets criteria for subject withdrawal or treatment discontinuation as per protocol guidelines.

End of treatment assessments below will be performed within 7 days after decision to end treatment. If the date to end treatment is determined later than 14 days from last study drug treatment, then it can be combined with the 30-day visit outlined in 6.8.2.

- Adverse event assessment
- Concomitant medication collection
- Complete physical exam, including neurological exam, and KPS assessment
- Vital signs: weight, temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase and lipase.
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein
- Thyroid stimulating hormone (TSH), free T4
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential

6.8 Post-Treatment Procedures

In this study, survival is a key endpoint. In addition, there is potential risk for delayed immune-related toxicities with study drug. **Therefore, post study follow-up is of critical importance and is essential to preserving subject safety and the integrity of the study**.

Subjects who discontinue study drug must continue to be followed for collection of outcome and/or survival follow-up data as required below until death or the conclusion of the study.

6.8.1 **Adverse Event Follow-up Post-Treatment:**

All participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 30 days of last study drug. Assessments may continue for ongoing reportable adverse events. Participants removed from protocol therapy for unacceptable adverse events will be following until resolution or stabilization of the adverse event.

6.8.2 **30-day and 90-day post-drug visits:**

A site visit is to be performed at 30 days (±7 days) and 90 days (±14 days) after the last study drug is given, unless the subject is unable to travel due to deteriorating medical condition, due to the potential risk for delayed immune-related toxicities. The visit will include the safety procedures detailed below:

- Adverse event assessment.
- Concomitant medication collection
- Symptom-directed physical exam and KPS assessment.
- Vital signs: weight, temperature, resting blood pressure, pulse and respiration rate.
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential
- *Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase and lipase.
- *Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count.
- *Thyroid stimulating hormone (TSH), free T4

  *For the 90-day post-drug visit, serum chemistry, hematology, TSH, and free T4 are required only if study-related toxicity persists.

6.8.3 **Extended Follow-up Post-Treatment:**

Following the 90-day post-drug visit, all subjects will be contacted every 3 months (±14 days) to assess for study-treatment related toxicities, initiation of any new anti-cancer treatments, and survival status. Contact may be performed by a site visit, telephone contact, e-mail, or mail. The date of death, initiation of any new anti-cancer treatments and date of last contact should be documented if this information is available. All subjects will be followed post-treatment until death, withdrawal of permission to record at least survival data, or subject is lost to follow-up.

If a subject withdraws permission to record at least survival data after coming off treatment, this must be documented along with the date the subject withdraws permission as per details in Section 6.12.

Subjects will be considered lost to follow up if no medical records are available to be reviewed and two phone calls each to the subject and then the subject's next-of-kin (if the subject does not respond) are not returned over two consecutive 3 month periods.

6.8.4 **Lost to Follow-up**

All reasonable efforts must be made to locate subjects to determine and report their ongoing status. This includes the subject's next-of-kin if the subject does not respond. Lost to follow-up is defined by the inability to reach the subject and then after a minimum of two documented phone calls, faxes, or emails in a 3 month period over two consecutive 3 month periods, a lack of response by subject to one registered mail letter, and if no medical records are available to be reviewed.

All attempts should be documented in the subject's medical records. If it is determined that the subject has died, the site will use permissible local methods to obtain the date and cause of death. The site staff will consult publicly available sources, such as public health registries and databases, in order to obtain updated contact information. If after all attempts, the subject remains lost to follow-up, then the last known alive date as determined by the investigator should be reported and documented in the subject's medical records.

6.9 Permanent Discontinuation of Study Treatment

Subjects MUST discontinue investigational product (and non-investigational study treatment at the discretion of the investigator) for any of the following reasons:

- Subject's request to stop study treatment
- Any clinical adverse event (AE), laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Termination of the study by the Sponsor or Bristol-Myers Squibb (BMS)
- The subject becomes pregnant. If subject becomes pregnant, the investigator must immediately (within 24 hours of awareness of the pregnancy) notify the Sponsor or designee of this event.

All subjects who discontinue study drug should comply with protocol specified follow-up procedures as outlined in Section 6.10. The only exception to this requirement is when a subject withdraws consent for all study procedures including post-treatment study follow-up or loses the ability to consent freely (i.e., is imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness).

If study drug is discontinued prior to the subject's completion of the study, the reason for the discontinuation must be documented in the subject's medical records and entered on the appropriate case report form (CRF) page.

6.10 Withdrawal of Subjects

6.10.1 **When and How to Withdraw Subjects**

A subject has the right to voluntarily discontinue study treatment or withdraw from the study at any time, for any reason, and without repercussion. The investigator and sponsor have the right to discontinue a patient from study treatment or withdraw a patient from the study at any time.

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

- Subject withdrawal of consent at any time
- Disease progression
- Intolerable toxicity
- Any medical condition that the investigator or sponsor determines may jeopardize the patient's safety if he or she continues in the study or continues treatment with study drug.
- The investigator or sponsor determines it is in the best interest of the patient

- Failure of subject to adhere to protocol requirements (e.g., not complying with protocol required visits, assessments, and dosing instructions)
- Pregnancy
- Meeting of another study withdrawal or discontinuation criterion
- Death
- Study termination by Sponsor

Subjects who request to discontinue study drug will remain in the study and must continue to be followed for protocol specified follow-up procedures. The only exception to this is when a subject specifically withdraws consent for any further contact with him/her or persons previously authorized by subject to provide this information. Subjects should notify the investigator of the decision to withdraw consent from future follow-up in writing, if possible. 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 drug only or also from study procedures and/or post treatment study follow-up, and entered on the appropriate CRF page. The date the participant was removed, must be documented. In the event that vital status (whether the subject is alive or dead) is being measured, publicly available information should be used to determine vital status only as appropriately directed in accordance with local law.

6.11 Research Specimen Procedures

- See Appendix 6 for Background and Rationale of Research Correlative Studies.
- Refer to the study procedures above for details on timing of research specimen collections.

Note: This study involves collection of **required archival tumor tissue and blood specimens for research** as well as collection of a small amount **fresh surgical specimen** (equivalent to amount required for a stereotactic needle biopsy), if available, for short-term organotypic tumor spheroid culture and flow-cytometry-based immunoprofiling, and an ***optional* on-study or post-study tumor specimen** if a biopsy is required during or after protocol therapy.

All patients will be given the option to have **leftover research specimens banked for future research** after study completion and completion of protocol-defined research studies at the time of informed consent. See Section 9.1.1 for details on confidentiality and banking of leftover research samples.

6.11.1 **Required Archival Tumor Specimen for Research**

Archival tumor tissue from a previous surgical resection that demonstrates histopathological evidence of glioblastoma (GBM, WHO grade IV) or gliosarcoma as specified in the Eligibility Criteria must be submitted.

A paraffin-embedded or frozen tumor-tissue block with a minimum of 1 cm^2 surface area of viable tumor is required. The tumor block should contain at least 20% viable tumor, i.e., a representative H&E slide should show that at least 20% of the specimen contains viable tumor.

If a tumor block cannot be submitted, then twenty (20) unstained 5-micron slides (preferably 10 slides from two different tumor blocks from the same surgery) from the tumor specimen must be submitted.

Send archival tumor specimens for research to:

Matija Snuderl, MD
Department of Pathology, NYU School of Medicine
560 First Avenue
Tisch Hospital HW 451
New York, NY 10016

Phone: 646-501-5281
Fax: 212-263-7916
Pager: 917-205-5543
Email: Matija.Snuderl@nyumc.org

6.11.2 Required Research Blood Samples

Research blood samples will be taken at only 2 time points:

Main Study Cohort: **Pre-dose C1D1** (up to 3 days prior to C1D1) and at the **C2D1 visit (end of week 6)**.

Surgical Study Cohort: **Pre-dose C1D-29** (up to 3 days prior to C1D-29) and at the **C1D15 visit (end of week 6)**.

All subjects will have the below research blood samples collected. For details on the timing of specimen collection, refer to the study procedures.

Whole blood for research will be collected from all subjects as described below:

- NYUSoM subjects: A total of 40 mL (four 10 mL purple top EDTA tubes) of whole blood will be drawn and immediately sent to the below sites:
    - One (1) 10 mL tube will be sent to the CBRD at NYUSoM for acquisition of whole blood, buffy coat, and plasma samples.
    - Three (3) 10 mL tubes will be sent to the IMC at NYUSoM and immediately processed for isolation of PBMCs.

6.11.3 Fresh Surgical Tumor Specimen (if available)

After informed consent, if subjects undergo tumor resections or biopsies prior to treatment initiation, during the study period, or after progression on study drug, a small amount fresh surgical specimen (less than 0.5 cm^2, approximately equivalent to amount required for a stereotactic needle biopsy) will be collected for short-term (6-8 days) organotypic tumor spheroid culture and for dissociation into single cell suspensions for flow-cytometry-based immunoprofiling. Fresh tumor specimen will only be collected after verifying that enough tumor sample for diagnosis and clinical care is available. All tumor material collected will be used for these specific purposes only. Tumor sample will be collected in medium (DMEM) in a sterile cryotube and immediately transported on ice to Dr. Kwok-Kin Wong's laboratory at NYU Langone for immediate processing.

6.11.4 Optional Tumor Biopsy for Research

If subjects undergo tumor resections or biopsies during the study period after treatment initiation, or after progression on study drug, a tumor specimen will be collected for research purposes if the subject provides consent at the time of informed consent.

A section of frozen tumor or a FFPE block (surface area of 1 cm^2 containing at least 20% viable tumor, as described above in Section 6.8.2) from the tumor surgery is preferred. If a frozen tumor specimen or a tumor block cannot be provided, then 20 unstained 5-micron slides (preferably 10 slides from two different tumor blocks from the same surgery) from the tumor block should be sent.

Send optional on-study or post-treatment tumor samples to:

Matija Snuderl, MD
Department of Pathology, NYU School of Medicine
560 First Avenue
Tisch Hospital HW 451

New York, NY 10016
Phone: 646-501-5281
Fax: 212-263-7916
Pager: 917-205-5543
Matija.Snuderl@nyulangone.org

7 Statistical Plan

7.1 Background for Statistical Plan

MGMT unmethylated, elderly and poor functional status newly diagnosed GBM patients treated on recent randomized clinical trials with regimens including radiotherapy, temozolomide or bevacizumab have median survivals in the range of 6-12 months, with one study that did not enroll patients with unresectable (biopsy-only) tumors[5] which carry poorer prognosis reporting 14 months.[3,4,7,57,80-82] With standard chemoradiation, the fraction of patients alive at 1 and 2 years for *MGMT* unmethylated patients aged 70 or less with good functional status are 50% and 6%.[7] The 1 year OS proportion for elderly and poor prognosis patients ranges from 21-32%.[57,80-82]

With regards to hypofractionated radiotherapy in newly diagnosed GBM, the IAEA randomized trial treated elderly and poor functional status GBM patients aged ≥50 with hypofractionated RT (5 fraction) and the median OS was 8 months.[57] In recent years, other small phase I and II studies have reported short course RT to have similar efficacy similar to standard treatment regimens in selected newly diagnosed GBM patients, although these studies did not stratify by *MGMT* methylation status.[52-56,68] Median OS in these *MGMT* non-stratified studies ranged from 15 to 20 months.

Based on these historical measures, a 12 month (1yr-OS) of 35% will be considered non-promising in *MGMT* unmethylated, newly diagnosed GBM subjects; an increase in the 1yr-OS to 60% will be considered promising.

Most recently, a phase II randomized multicenter clinical study in patients with recurrent glioblastoma suggest that treatment with the PD-1 inhibitor pembrolizumab results in significantly prolonged overall survival when given neoadjuvantly (prior to tumor re-resection) as compared to pembrolizumab adjuvantly (after tumor re-resection). This increase in overall survival was associated with upregulated T cell-activation and Interferon-γ-gene expression signatures and downregulation of cell-cycle related gene expression signatures in tumor cells. In this study, interferon- and T cell-pathway induction was seen in 9 of 14 tumors (64%) from patients who received neoadjuvant pembrolizumab compared to 5 of 15 tumors (33%) from patients who received pembrolizumab adjuvant-only.[64]

Based on these measures, a T-cell pathway induction rate of 33% will be considered non-promising; an increase in the T-cell pathway induction rate to 64% will be considered promising.

7.2 Statistical Methods and Sample Size Determination

7.2.1 **Safety**

The Safety Lead-In portion of the study will include the first 6 subjects enrolled on the Main Study Cohort. The exact number of patients enrolled in the entire study will depend on the number of protocol-defined DLTs observed and the possibility of opening additional cohorts at modified nivolumab and/or ipilimumab prescriptions as described in Section 6.1.1 (Safety Lead-In). Data for the Safety Lead-In will be summarized using descriptive statistics.

Adverse events will be graded using CTCAE version 4.03. Safety summaries will be reported for both the Safety Lead-In and the phase II trial. All subjects who received at least one dose of study drug and/or radiation will be used for the analysis of safety data in this study. Safety and tolerability will be assessed

by clinical review of all relevant parameters including adverse experiences (AEs), laboratory tests, and vital signs.

Additionally, specific immune-related adverse experiences (irAEs, as defined Section 6.2.1) will be collected and summarized in separate tables from other AEs by toxicity grade and will include the counts, percentage, and 95% CI.

Adverse events (specific terms as well as system organ class terms) and predefined limits of change in laboratory, and vital sign parameters will be summarized with descriptive statistics (counts, percentage, mean, standard deviation, etc.). Continuous measures such as changes from baseline in laboratory, and vital signs parameters will be summarized using descriptive statistics (mean, standard deviation, etc.) for baseline, on-treatment, and change from baseline values.

For toxicity monitoring statistical methods, see the Primary Safety Endpoints in Section 3.5.

7.2.2 **Main Study Cohort - Phase II trial design**

A single-stage design will be utilized to estimate overall survival,[83,84] with a hypothesized 1yr-OS of 60%=H_1 and 35%=H_0. We will enroll 24 patients; this design has a two-sided Type I error of 0.05 and a power of 80%.

For endpoint analyses, one year will be defined as 48 weeks (8 cycles) and two years will be defined as 96 weeks (16 cycles). Patients will be evaluated for radiographic responses by conventional contrast enhanced MRI every 8 weeks. Radiographic response assessments will be determined using RANO criteria for GBM.[90]

1yr-OS and radiographic response rates will be estimated with exact 95% confidence intervals; with 24 patients, a 95% CI will be no more than 0.4 units wide. OS will be determined from the time of treatment initiation until the time of death due to any cause, with OS being censored at last follow-up if the patient remained alive. Patients who were last known to be alive and progression-free will be censored at the latest disease assessment. The Kaplan–Meier curves will be used to summarize OS and to estimate the medians.

PFS is defined as the time between treatment initiation and first occurrence of disease progression or death, and will be censored at last follow-up if the patient remained alive without disease progression. PFS is defined as the time from the first dose of study treatment to the earlier of disease progression or death due to any cause. Patients who initiate alternate anticancer therapy in the absence of documented progression will be censored at the latest disease assessment prior to initiation of such therapy. Patients with no post-baseline disease assessments will be censored at the Cycle 1 Dose 1 date unless death occurred prior to the first planned assessment (in which case the death will be considered a PFS event). The Kaplan–Meier curves will be used to summarize PFS and to estimate the medians.

Overall response rate (ORR) is defined as the proportion of patients who achieve best overall response of complete or partial response at any time after initiation of study treatment (prior to initiation of alternate anticancer treatment). Duration of response is defined as the number of months from the time criteria are first met for either CR or PR, until the first date that PD is objectively documented. The duration of objective response will be summarized descriptively using the Kaplan-Meier method.

7.2.3 **Phase II trial – Surgical Study Cohort**

A single-stage design will be utilized to estimate overall survival, with a hypothesized rate of T-cell pathway induction of 64%=H_1 and 33%=H_0. We will enroll 16 patients; this design has a two-sided Type I error of 0.05 and a power of 80%.[64]

7.2.4 **Biomarker Analyses**

Correlative biomarker endpoints are exploratory and hypothesis generating. The correlative studies are not powered to make a formal test of the prognostic power of any biomarker. We will use non-parametric statistics (e.g. Wilcoxon, Spearman's rank correlation coefficient, Fisher's Exact text) to test the association between patients that achieve 1yr-OS and those that do not and the fraction of predicted mutation-associated neoantigens, fraction of expressed mutation-associated neoantigens, and levels of tumor-infiltrating immune populations identified by RNA-Seq between.

For the circulating Treg and MDSC biomarker study, we summarize the distributions of levels at baseline and changes from baseline using descriptive statistics and graphical displays. Changes from baseline will be examined using Wilcoxon signed rank tests for paired measurements. Logistic regression will be used to predict 1yr-OS (binary) based on baseline levels and the changes from baseline. Optimal cutpoints for changes from baseline will be estimated for the separation of those patients who are alive at 1 year compared to those who are not using the Youden Index based on the receiver operating characteristic curve.

For the tumor tissue PD-L1 and TIL assays, we will use similar descriptive analyses and summary displays to assess whether expression of PD-L1 is associated with 1yr-OS. Additional analyses will combine all of these key biomarkers in logistic models to identify potential combinations of markers to predict 1yr-OS.

Imaging features of tumor cell death and inflammation will be defined as increased contrast-enhancement on conventional MRI, and a statistically significant elevation of mean tissue sodium concentration (TSC) by at least 25% on 23Na MRI.

Associations between other exploratory biomarker measures from peripheral blood or tumor and clinical outcomes will also be explored graphically, and further assessed as needed by methods such as, but not limited to, logistic regression, and characterized by appropriate statistics.

7.3 Subject Population(s) for Analysis

7.3.1 **Safety Lead-In Population**

Subjects are evaluable for DLTs if: 1) they experience a DLT at any time during the DLT evaluation period; or 2) in the absence of DLT have received at least one dose of nivolumab or ipilimumab, have completed RT per protocol, and have completed respective safety assessments without major violations over the entire DLT evaluation period. Patients who are not fully evaluable for DLTs during the DLT evaluation period of 40 days following Day 1 will be replaced.

7.3.2 **Phase II Trial Efficacy Analysis Population**

All subjects that receive at least 1 dose of study drug will be included in the primary analysis for the phase II study, including subjects in the Safety Lead-In treated at the same dose as the phase II subjects.

7.3.3 **Safety Analysis Populations**

All subjects who received at least one dose of study drug will be used for the analysis of safety data in this study. Safety summaries will be reported for both the Safety Lead-In and the phase II trial.

At least one laboratory or vital sign measurement obtained subsequent to at least one dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

7.3.4 Surgical Study Cohort Analysis Population

All subjects that receive at least 1 dose of study drug and undergo a craniotomy and tumor re-resection will be included in the safety and efficacy analysis for the Surgical Study Cohort Analysis.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

ADVERSE EVENT (AE)

An ***adverse event (AE)*** is defined as any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. An AE also includes any worsening (i.e., any clinically significant change in frequency and/or intensity) of a pre-existing condition that is temporally associated with the use of the study drug.

Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

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.)

SERIOUS ADVERSE EVENT (SAE)

Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- 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 or prolongs in-patient hospital stay (In-patient hospitalization is defined as admission to a hospital or an emergency room for longer than 24 hours. Prolongation of existing hospitalization is defined as a hospital stay that is longer than was originally anticipated for the event, or is prolonged due to the development of a new AE as determined by the investigator or treating physician. Hospitalization or prolongation of existing hospitalization due to the progression of

underlying malignancy will not be considered an SAE, if it is clearly consistent with the typical progression pattern of the underlying cancer (see **NOTE** below).
- results in persistent or significant disability or incapacity (substantial disruption of one's ability to conduct normal life functions)
- a congenital anomaly or birth defect
- an important medical event - Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious. Potential drug induced liver injury (DILI) is also considered an important medical event (see below for the definition of potential DILI).
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug.
- Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

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

NOTE: Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Exceptions: Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:
- 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)
- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization 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.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs.

NON-SERIOUS ADVERSE EVENT

All adverse events that do not meet any of the criteria for **Serious Adverse Event** (defined above) should be regarded as ***non-serious adverse events***.

Progression of underlying malignancy

Progression of underlying malignancy will not be considered an AE if it is clearly consistent with the typical progression pattern of the underlying cancer (including time course, affected organs, etc.). Hospitalization or death due solely to manifestations consistent with typical progression of underlying malignancy will not be considered a SAE. Clinical symptoms of progression may be reported as AEs if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

If there is any uncertainty about an AE being due only to progression of the underlying malignancy, it should be reported as an AE or SAE as outlined in Section 8.4.

Adverse Event Reporting Period
All Adverse Events (AEs) and Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study and for a minimum of 100 days after the last dose of nivolumab and 90 days after the last dose of ipilimumab must be reported per the guidelines in Section 8.4, whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (e.g., an optional tumor biopsy).

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.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
All laboratory test results captured as part of the study should be recorded following institutional procedures.

A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. more frequent follow-up assessments, further diagnostic investigation, specific corrective therapy, etc.
- The abnormality leads to a change in dosing (outside of protocol-stipulated dose adjustments)
- The abnormality leads to discontinuation from the study, significant additional concomitant medication, or other therapy
- The laboratory test result is clinically significant or meets the definition of an SAE

Contact the sponsor in the event the investigator feels that an abnormal test finding should be reported as an AE, although it does not meet any of the above criteria.

Test results that constitute SAEs should be documented and reported as such per the guidelines outlined in Section 8.4.

Repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

Evaluation of severity of laboratory abnormalities will be assessed according to the scale outlined in Section 8.2.1.

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 8.4 for reporting details).

Potential drug induced liver injury (DILI) is defined as:

1) AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)

AND

1) Total bilirubin > 2 times 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.

Pregnancy
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 (within 5 months after the last dose of study drug for a female subject and within of 7 months after the last dose of study drug for a male subject), the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for participant).

The investigator must immediately notify Worldwide.Safety@bms.com of this event via the **Pregnancy Surveillance Form** [provided upon request from BMS] in accordance with SAE reporting procedures. The investigator must also immediately notify the Sponsor/Overall PI of the event.

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 on the Pregnancy Surveillance Form.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. 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.

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.

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.

Expected Adverse Events for Nivolumab and Ipilimumab
For the purpose of regulatory reporting requirements during clinical development, the following AEs will be considered as **expected** and meet the threshold of causal association (based on comprehensive medical evaluation considering the mechanism of action and temporal relationship after excluding other possible etiologies) defined by nivolumab and ipilimumab based on safety data from completed and ongoing clinical studies:

- Infusion-related reactions including drug hypersensitivity reactions
- Immune-mediated adverse reactions such as immune-mediated colitis, immune-mediated hepatitis including autoimmune hepatitis, immune-mediated thyroid disorders including hyperthyroidism, hypothyroidism, thyroiditis and autoimmune thyroiditis, immune-mediated pneumonitis, immune-mediated skin reactions including rash, pruritus, rash generalized, rash maculo-papular, erythema, pemphigoid, other immune-mediated reactions including myocarditis, adrenal insufficiency, uveitis, iritis, myositis.

Expected Adverse Events for Radiation Therapy
Development of edema in the brain is common following brain radiation therapy and may be asymptomatic (solely a radiographic finding) or associated with mild, moderate or severe symptoms. Therefore, brain edema will be considered an expected event related to study therapy (RT). Additionally, alopecia, fatigue, transient worsening of baseline neurological symptoms, seizures, skin irritation, neurocognitive dysfunction and cerebral radiation necrosis are all expected adverse events associated with radiation therapy.

8.2 Evaluation of Severity and Causality

8.2.1 Evaluation of Severity

The severity of AEs (including test findings classified as AEs) will be graded using the current version of the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) grading system. Adverse events not listed in the NCI-CTCAE, will be graded according to the following scale:

1 (Mild):	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2 (Moderate):	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
3 (Severe):	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4 (Life-threatening):	Life-threatening consequences; urgent intervention indicated.
5 (Death):	Death related to AE.

* Instrumental Activities of Daily Living (ADL) refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

8.2.2 Evaluation of Causality

Relationship of AEs to Study Drug:
The relationship of AEs to study drug will be assessed by the investigator, and will be a clinical decision based on all available information. The following question will be addressed:

Is there a reasonable possibility that the AE may have been caused by the study drug?

The possible answers are:

Not Related: There is no reasonable possibility that the event may have been caused by the study drug

Related: There is a reasonable possibility that the event may have been caused by the study drug.

The sponsor will request information to justify the causality assessment of SAEs, as needed.

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.

Appendix 5 lists factors to consider in assessing the relationship of AEs to nivolumab, ipilimumab, infusion procedures, radiation therapy, combination treatment, or study procedures.

8.3 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period and for a minimum of 100 days after the last dose of nivolumab and 90 days after the last dose of ipilimumab must be recorded. Following the subject's written informed consent to participate in the study, all AEs and SAEs, whether related or not related to study drug, are to be continuously 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.

The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

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.

8.4 Reporting of Serious Adverse Events and Unanticipated Problems

The investigator (or designee) will seek information on AEs at each patient contact, and record all AEs that occur from the time the informed consent is signed until 30 days after the end of study treatment. CTCAE version 4.03 terms should be used.

All AEs after initiation of study treatment and until 30 days after the last study treatment, regardless of relationship to study treatment, will be reported. Additionally, any SAE or other AE of concern that the investigator believes may be related to study treatment and that occurs later than 30 days after last study treatment should be reported. Information for any nonserious AE that starts during the treatment period or within 30 days after last treatment will be collected from the time of the event until resolution of the event, or until the patient's last study visit, whichever comes first. Serious adverse event information will be collected until the event is considered chronic and/or stable.

Study treatment includes nivolumab, ipilimumab and radiation therapy.

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others (see definitions, section 8.1).

Note: Immediately reportable criteria must be reported to the IRB at each annual review.

Serious adverse event reporting will begin in conjunction with the date of informed consent. Any SAEs occurring prior to study drug administration that the investigator believes may have been caused by a protocol procedure must be reported immediately to the Sponsor or its designee and recorded on the case report form.

All fatal or life-threatening adverse events must be immediately reported to the Sponsor by telephone or e-mail. Within 24 hours of the event, the Serious Adverse Event (SAE) Form supplied by NYUSoM must be faxed to the Sponsor (Overall PI), who must then inform the NYUSoM IRB, PCC CTO, and DSMC within 24 hours of the event whether full information regarding the event is known or not. Additional follow-up by the investigator will be required if complete information is not known. De-identified source documentation of all examinations, diagnostic procedures, etc. which were completed with respect to the event should be included with the SAE form. Care should be taken to ensure that the patient's identity is protected and the patient's identifiers (as assigned at the time of study enrollment) are properly mentioned on any copy of source document provided to the Sponsor. For laboratory results, include the laboratory normal ranges.

In case of accidental or intentional overdose of study drug (nivolumab and/or ipilimumab), even if asymptomatic or not fulfilling a seriousness criterion, the overdose is to be reported to the Sponsor immediately (within 1 working day) using the AE and SAE forms supplied by NYUSoM. Overdose of study drug is defined in Section 8.1.

All serious adverse events (SAEs) will be evaluated by the DSMC If meeting the requirements for expedited reporting, the Sponsor will report the adverse event to all regulatory authorities with jurisdiction over ongoing trials with the study drug and to all other investigators involved in clinical trials with the study drug. The investigator is responsible for reporting all SAEs to the appropriate IRB, DSMC, and FDA.

For Narrative Reports of Safety Events
If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.4.1 Investigator Reporting: Notifying the Study Sponsor, NYUSoM IRB, Perlmutter Cancer Center Clinical Trials Office (PCC CTO), and BMS

The following describes events that must be reported to the study **Sponsor** in an expedited fashion.

Initial Report: within 24 hours of awareness of the event:
The following events must be reported to the study sponsor by telephone within 24 hours of awareness of the event:

- Unanticipated problems related to study participation,
- Serious adverse events, regardless of whether they are unexpected.
- Overdose of Study Drug, defined in Section 8.1.
- Pregnancy: Although pregnancy is not considered an AE, it is the responsibility of the investigator to report to the Sponsor (or designee), by telephone within 24 hours of identification, any pregnancy occurring in a female subject or female partner of a male subject, during the study or within 5 months after the last dose of study drug for a female subject or within 7 months after the last dose of study drug for a male subject. Any complication of pregnancy affecting a female subject or female partner of a male subject, and/or fetus and/or newborn must be reported as an SAE. All pregnancy outcomes will be followed as described in Section 8.1. Pregnancies must also be reported on a Pregnancy Surveillance Form to BMS immediately as described in Section 8.1.
- Exposure during Pregnancy or Breastfeeding (even if not associated with an adverse event)
- Occupational exposure (even if not associated with an adverse event)
- Potential drug-induced liver injury (DILI): These events are considered important medical events and should be reported as SAEs. See Section 8.1 for DILI definition.

In the event the investigator is informed of an SAE that occurs after 30 days after the last dose of study treatment, only those SAEs or other AEs of concern deemed by the investigator to be related to study treatment will be reported to the sponsor. The investigator should make every effort to obtain follow-up information on the outcome of a treatment-related SAE until the event is considered chronic and/or stable.

Additionally, an **FDA Form 3500 (MEDWATCH Form)** must be completed by the investigator and faxed to the study **Sponsor/Overall PI** within 24 hours. The investigator shall maintain a copy of the MEDWATCH Form on file at the study site or can be obtained from the FDA website: http://www.fda.gov/safety/medwatch/howtoreport/downloadforms/default.htm.

Sponsor contacts (NYUSoM):

NYUPCCsafetyreports@nyumc.org

AND

Sylvia Kurz, MD, PhD
Laura and Isaac Perlmutter Cancer Center
NYU School of Medicine
240 East 38th Street, 19th Floor

New York, NY 10016
Phone: 212-731-6267
Fax: 646-754-9696
Email: Sylvia.Kurz@nyulangone.org

AND

PCC Assigned Medical Monitor

Follow-up report: within 48 hours of awareness of the event:
As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated device event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Unanticipated Problem form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing unanticipated adverse device effects shall be provided promptly to the study sponsor.

All report forms must be signed and dated by the Principal Investigator. If the Principal Investigator is not available at the time of the initial report, then the form can be submitted by a Sub-Investigator. This form should be reviewed by the Principal Investigator, who must sign/date the initial report upon their return.

New information available after 48 hours of initial event: within 24 hours of awareness of new information
If new information about a previously reported event becomes available 48 hours after the initial awareness of the event, this new Information should be reported within 24 hours of awareness of the new information. Any new follow-up information that is received or that the investigator is newly made aware of after the initial 48 hour reporting period should be reported within 24 hours from the time of awareness of the new information.

Other Reportable events:

- **Deviations from the study protocol**
  Deviations from the protocol must receive both Sponsor and the investigator's IRB approval before they are initiated. Any protocol deviations initiated without Sponsor and the investigator's IRB approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the Sponsor and to the investigator's IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation.
- **Withdrawal of IRB approval**
  An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but ***no later than 5 working days*** of the IRB notification of withdrawal of approval.

8.4.2 Investigator Reporting: Notifying the IRB

Federal regulations require timely **reporting by investigators to their local IRB** of unanticipated problems posing risks to subjects or others. The IRB requirements reflect the current guidance documents released by the Office of Human Research Protection (OHRP), and the Food and Drug Administration (FDA) and are respectively entitled "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events" and "Guidance for Clinical Investigators, Sponsors, and IRBs: Adverse Event Reporting-Improving Human Subject Protection." The following describes the NYUSoM IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record. The NYU IRB address is:

NYUSoM IRB

1 Park Avenue, 6th Floor
New York, NY 10016

Report Promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events*** **that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

At the time of each annual review any protocol deviations stated above, such as dose-reductions (even if done in accordance with protocol guidelines) must be reported to the IRB.

8.4.3 Sponsor Reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as **IND safety reports**. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days*** *(via telephone or facsimile report)*
  Any study event that is:
  – associated with the use of the study drug
  – unexpected,
  – fatal or life-threatening

- ***Within 15 calendar days*** *(via written report)*
  Any study event that is:
  – associated with the use of the study drug,
  – unexpected, and
  – serious, but not fatal or life-threatening
  -or-
  – a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  – suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A (MEDWATCH Form, obtained from the FDA website: http://www.fda.gov/safety/medwatch/howtoreport/downloadforms/default.htm), or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3. The contact information for submitting IND safety reports is noted below:

Email: NYUPCCsafety@nyumc.org
Tel: 212-263-2748

8.4.4 Sponsor Reporting: Notifying Participating Investigators

It is the responsibility of the study sponsor to notify all participating investigators of any adverse event that meets the FDA 15-day reporting requirement criteria as note above in Section 8.4.3. The same materials and timeline used to report to the FDA are used for notifying participating investigators.

8.4.5 Sponsor Reporting: Notifying BMS

SAEs, whether related or not related to study drug, and pregnancies must be reported by the Sponsor/ Overall PI to BMS within 24 hours of awareness of the event. SAEs must be recorded on BMS or an approved form; pregnancies must be reported on a Pregnancy Surveillance Form.

SAEs that occur following the subject's written consent to participate in the study through 100 days of the last dose of nivolumab and 90 days of the last dose of ipilimumab must be reported by the Sponsor to 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 biopsy).

Following the subject's written consent to participate in the study and for a minimum of 100 days of the last dose of nivolumab and 90 days of the last dose of ipilimumab, all AEs 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 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 by the Sponsor within 24 hours to BMS (or designee) using the same procedure used for transmitting the initial SAE report.

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

BMS Reporting Contacts:

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: +1 609-818-3804

8.5 Stopping Rules

For the Safety Lead-In, see Section 6.1.1 for detailed rules for continuing enrollment, dose decisions, and study discontinuation. In summary, once the Safety Lead-In cohort has filled enrollment, enrollment will stop and subjects will be observed for 40 days for dose-limiting toxicities (DLTs). At the end of the DLT period, the safety data will be reviewed by the Sponsor and the NYU DSMC. Continuation of enrollment will occur once all of the subjects have completed the Day 40 safety assessments and the final safety review decisions, including whether the study should be discontinued or proceed to phase II, have been made.

For the Phase II trial, stopping rules for unexpected toxicity are outlined in see Section 3.5, Primary Safety Endpoints.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see **Section 10 Auditing, Monitoring and Inspecting**). Adverse events are evaluated regularly by the principal investigator in conjunction with the research team. The NYU Data Safety and Monitoring Committee (DSMC) will review the study quarterly. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 **Data and Safety Monitoring Committee**

This investigator-initiated study will be monitored by the Data Safety Monitoring Committee (DSMC) of the New York University (NYU) Perlmutter Cancer Center (PCC). The DSMC operates based on the 2014 National Cancer Institute approved Charter. It is an existing and multidisciplinary committee (consisting of clinical investigators/oncologists, biostatisticians, nurses, and research administration staff knowledgeable of research methodology and design and in proper conduct of clinical trials) that is responsible for monitoring safety, conduct and compliance in accordance with protocol data monitoring plans for clinical trials conducted in the NYUSoM Perlmutter Cancer Center that are not monitored by another institution or agency. The DSMC reports to the Director of the NYUSoM PCC. Per the NYU PCC Institutional Data Safety and Monitoring Plan, this phase 2 trial will be monitored by DSMC quarterly (from the date the first patient is enrolled), at the end of the Safety Lead-In, subsequent phase II activation, and at the completion of the study prior to study closure. This review includes accrual data, subject

demographics and adverse events. Accrual to the phase II will be held until real-time review of the toxicity from the Safety Lead-In has occurred to assure no defined DLTs have occurred prior to proceeding. Additional reviews can be scheduled based on SAE reports, investigator identified issues, external information, etc. The DSMC will review safety data every 3 months.

9 Data Handling and Record Keeping

9.1 Confidentiality

The study team will maintain clinical and laboratory data in a designed manner to ensure patient confidentiality. All study personnel have passed human subject protection courses. If applicable, tissue samples sent to collaborators outside of NYUSoM will only be labeled with an assigned protocol-subject identification number without patient identifiers. Systems used for electronic data capture are compliant with HIPAA and applicable local regulatory agency guidelines. All documents are kept in strictly confidential files and are only made accessible for specific study personnel, CTO quality assurance specialists, and authorized representatives of regulatory agencies as described in the informed consent document.

9.1.1 **Leftover Research Samples (Tissue and Blood)**

At the time of informed consent to participate in this trial, patients will have the option to allow all research samples (archival tumor sample, research blood samples, and optional tumor biopsy sample) remaining after completion of the study and protocol-specified correlative biomarker research to be banked for future research studies. Future studies may include, but are not limited to, genetic, epigenetic, and molecular studies with the overall goal of correlating any scientific findings with the patients' outcome to protocol therapy. Leftover samples will be stored in a repository in the NYUSoM Center for Biospecimen Research and Development (CBRD) and labeled with an assigned protocol-patient identification number without subject identifiers. The assigned protocol-patient identification numbers will be stored in a central database on a password-protected NYUSoM server. This central database will contain the key to decoding assigned protocol-patient identification numbers used for sample labeling and patient's identifiable medical information (PHI). Only the Overall PI will have the linking key to the subject identifiers.

For each leftover research sample, key clinical information including, but not limited to, gender, age at diagnosis, tumor location, prior treatment and histology will be abstracted from the medical record and recorded with the assigned protocol-patient identification number on a separate database from the central database containing the identifiable PHI. This de-identified database will also be on a password-protected NYUSoM server and will be administered by the overall PI. Only the Overall PI and IRB-approved study investigators with permission from the Overall PI will have access to this de-identified database.

The de-identified samples and data may be made available to researchers at NYULH (NYU Langone Health), NYUSoM (NYU Langone Medical Center), NYU SoM (NYU School of Medicine), and other NYUSoM affiliates, as well as researchers outside of NYU. De-identified samples may be shared with for-profit companies that are working with NYULH, NYUSoM, NYU SoM or other NYULH affiliate researchers on a specific IRB-approved research project. De-identified specimens may also be made available to other researchers through research organizations whose mission focuses on the acquisition, authentication, production, preservation, development and/or distribution of data and materials for research in the life sciences, such as The Cancer Genome Atlas, a collaboration between the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI).

Patients not agreeing to allow their leftover samples/data to be stored for future use will be able to fully participate in the study with no restrictions. The central database and de-identified database maintained for at least 5 years after termination of the study.

Subjects can withdraw their samples/data from future use at any time. The subject may contact the Study Sponsor/Overall PI or designee to express his or her wish to withdraw leftover samples from storage.

9.2 Confidentiality and HIPAA

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.3 Data and Source Documentation

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

An electronic database capture system will be created to record the data for this trial. Research coordinators will input clinical trial data into the database. This database is password protected and only the PI, assigned research coordinator, and CTO quality assurance specialists will have access to the database. DataCore, a core resource of the institution, will provide the primary data collection instrument for the study. All data requested in the system must be reported. All missing data must be explained. The quality assurance specialists will monitor this trial every 4-6 weeks for data entry accuracy.

Source documentation should be consistent with data entered into the electronic database. Relevant source documentation to be reviewed by the DSMC throughout the study includes:

1. Baseline measures to assess pre-protocol disease status
2. Concomitant medications
3. Treatment records
4. Adverse events

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan detailed below. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit. A risk-based, data-driven monitoring approach will be used to verify data for this trial which will also include a centralized review of data for quality, trends, consistency and general safety review. A quality assurance specialist will make regularly scheduled trips to the investigational site to review the progress of the trial, study data and site processes. At each visit, the monitor will review various aspects of the trial including, but not limited to: screening and enrollment logs; compliance with the protocol and with the principles of Good Clinical Practice; completion of case report forms; source data verification; study drug accountability and storage; facilities and staff.

During scheduled monitoring visits, the investigator and the investigational site staff must be available to meet with the quality assurance specialist in order to discuss the progress of the trial, make necessary corrections to case report form entries, respond to data clarification requests and respond to any other trial-related inquiries of the monitor. In addition to on-site monitoring visits, the Sponsor and/or representatives will also be routinely reviewing data. Any queries identified through this review will be managed within the systems established for query resolution and tracking. Inquiries related to study conduct, which require further information or action will be discussed within the study team for appropriate and documented escalation plans. It is expected that response to data clarification requests and other trial-related inquiries will occur throughout the course of the study through regular communication with the site monitor, the Sponsor or representatives, and review/entry of data into the electronic study database.

At any time during the course of the study, representatives of the FDA and/or local regulatory agencies may review the conduct or results of the study at the investigational site. The investigator must promptly inform BMS of any audit requests by health authorities, and will provide BMS with the results of any such audits and with copies of any regulatory documents related to such audits.

In accordance with HIPAA and associated privacy regulations, a patient's authorization to use personal identifiable health information may be required from each patient before commencement of research activities. This authorization document must clearly specify what parties will have access to a patient's personal health information, for what purpose and for what duration.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

At the NYU Perlmutter Cancer Center, all investigator-initiated protocols are subject to a standardized data and safety monitoring, which includes scientific peer review, IRB review and DSMC review as well as internal auditing.

The review of AEs and trial conduct for this trial occurs at several levels:

(1) Principal Investigator: Adverse events are evaluated monthly by the principal investigator in conjunction with the research nurses, data manager and research team.

(2) DSMC, quarterly

(3) Institutional Review Board (IRB): An annual report to the IRB is submitted by the trial PI for continuation of the protocol. It includes a summary of all AEs, total enrollment with demographics, protocol violations, and current status of subjects as well as available research data.

(4) In addition, the quality assurance unit will provide extensive monitoring, including real-time review of all eCRFs to ensure completeness and compliance with the protocol. A first subject audit will be conducted within 4 weeks of enrollment. Additionally, monitoring of this trial will progress every 4-6 weeks thereafter to verify adherence to the protocol; the completeness, accuracy and consistency of the data; and adherence to ICH Good Clinical Practice guidelines.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice (GCP), and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent. The consenting process and documentation will follow Standard Operating Procedures of the NYUSoM PCC CTO.

12 Study Finances

12.1 Funding Source

Funding for conducting the trial will be provided by BMS. The investigational agents (nivolumab and ipilimumab) will be provided to patients enrolled on this study by subject.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that

has been reviewed and approved by the study sponsor prior to participation in this study. All NYUSoM investigators will follow the applicable University conflict of interest policies.

12.3 Subject Stipends or Payments

No patient or subject will receive payments or stipends for participation in this research study.

13 Publication Plan

NYUSoM fulfills its commitment to publicly disclose the results of studies.

The overall Principal Investigator, Sylvia Kurz, M.D., Ph.D., holds the primary responsibility for publication of the study results. The co-investigators of this study must first obtain approval from Dr. Kurz, the primary responsible party for publication, before any information collected in this trial can be used or passed on to a third party. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the overall Principal Investigator for the purposes of performing the study, will be published or passed on to any third party without the consent of the study overall Principal Investigator. Any investigator involved with this study is obligated to provide the overall Principal Investigator with complete test results and all data derived from the study.

BMS has no objection to publication by the study overall Principal Investigator of any information collected or generated by the study overall Principal Investigator, whether or not the results are favorable to the investigational drug. However, to ensure against inadvertent disclosure of confidential information or unprotected inventions, Investigator will provide BMS an opportunity to review any proposed publication or other type of disclosure before it is submitted or otherwise disclosed.

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15 Appendix

15.1 Appendix 1: Schedule of Events for Main Study Cohort

STUDY DAY ►		Day -21 to -1 [2]	C1 D1[12]	C1 D8[16]	C1 D15 and D29	C2 D1	Week 8+ [27]	End of Rx[31]	30-Day Post-Drug [32]	90-Day Post-Drug [33]	Surv F/u [34]
Study Team Procedures											
Informed Consent [1]		X									
Demographics		X									
Inclusion/Exclusion Criteria [3]		X									
Medical/Disease History [4]		X									
MGMT testing [6]		X									
KPS Assessment [9]		X	X			X		X	X	X	
Concomitant Medications [10]		X	X		X	X		X	X	X	
Height and Weight [13]		X	X		X	X		X	X	X	
Vital Signs [14]		X	X	X	X	X		X	X	X	
Complete Physical Exam [15]		X	X		X[15]	X		X			
Directed Physical Exam [16]				X	X[16]				X	X	
Adverse Events [11]		X	X	X	X	X		X	X	X	
Survival								X	X	X	X
Laboratory/Cardiology Assessments											
Serum Chemistry [17]		X	X		X	X		X	X	X [33]	
Hematology [18]		X	X		X	X		X	X	X [33]	
T cells (CD3, CD4, CD8) [35]		X				X[35]					
Urinalysis [19]		X			X	X		X			
Coagulation [7]		X									
Hepatitis B and C [8]		X									
Pregnancy Test [20]		X	X			X		X	X	X	
TSH, free T4 [21]		X	X			X		X[19]	X	X [33]	
12-Lead ECG [22]		X									
Study Treatment											
Radiotherapy: 3 Gy x 15 fractions (45 Gy total) [24]				X							
Nivolumab IV [25]			X		X	X					
Ipilimumab IV [26]			X			X					
Imaging Assessments											
MRI Brain [27]		X					X[27]				
Chest X-ray [23]		X									
Research Procedures											
Archival tumor tissue [5]		X									
Research blood samples [28]			X			X					
Research MRI (sodium MRI) [29]		X					X[29]				
Surgical tumor specimen [36]		X[36]									
Tumor biopsy (optional) [30]								X			

One treatment cycle will be defined as 42 days

EXPLANATION OF SUPERSCRIPTS:

1. Informed Consent: Performed by MD attending only. Informed consent process to be fully document: e.g. prospective participant had sufficient time for deliberation, all questions were answered, treatment options provided by MD, full study reviewed including risks, and a copy of signed consent given to the participant. No study specific screening procedures may occur until after the informed consent process is complete. Informed consent may be obtained more than 21 days before the start of screening procedures. Assessments performed as part of standard of care that fall within the screening window but before informed consent is obtained may be used for screening, and need not be repeated for enrollment eligibility.
2. Screening will start within 21 days prior to initiation of study treatment (first dose of study drug).
3. Inclusion/exclusion criteria: source documentation providing investigator's confirmation that the participant had met all eligibility criteria must be available prior to registration.
4. All patients must have histopathological documentation of glioblastoma or gliosarcoma (WHO grade IV). Subjects must not have had any prior anti-tumor therapy for glioblastoma or gliosarcoma other than surgery. Medical history should also include review of any ongoing medical conditions and medical history pertaining to eligibility on study and involvement during study.
5. All patients will be required to provide a tumor block or 20 unstained slides from a tissue specimen that demonstrates glioblastoma or gliosarcoma (WHO grade IV). See study procedures for details.
6. *MGMT* testing: *MGMT* promoter methylation status must be determined by central testing at NYUSoM. Only subjects with unmethylated MGMT promoter confirmed by central testing are eligible. See study procedures for details of *MGMT* testing.
7. Coagulation – PT/INR, PT, PTT required at screening only.
8. Hepatitis B virus surface antibody, hepatitis B virus surface antigen, hepatitis B virus core antibody, and hepatitis C virus antibody at screening only.
9. Karnofsky Performance Status (KPS) assessment: See Appendix 2 for KPS scale.
10. Concomitant medication recording will be ongoing throughout the course of the study. Record concomitant medications from within 21 days before starting study treatment up to the 90-Day Post Drug Visit.
11. All adverse events must be monitored throughout the study via safety assessments, observation, and participant reporting and for 100 days after the last dose of nivolumab and 90 days after the last dose of ipilimumab.
12. Baseline (Pre-C1D1) assessments must be performed within 3 days prior to the first dose of study drug. Evaluations performed at screening that fall within 3 days of treatment initiation will not need to be repeated. The baseline labs do not need to re-meet the specific eligibility criteria, only meet criteria for dosing
13. Height is required only at screening. Weight can be taken within 3 days of scheduled study drug administration.
14. Vital signs include temperature, resting blood pressure, pulse, and respiration rate. When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection. At C1D1, vital signs will be collected prior to treatment, at the end of the infusion, and every 30 minutes for the first hour post-infusion (all VS timepoints ±10 minutes). Vital signs on subsequent treatment days of cycle 1 and all subsequent cycles will be assessed and documented prior to the infusion, and then 30 minutes (±10 minutes) after the completion of the infusion. During Radiation Therapy, limited vital signs (temperature, resting blood pressure, pulse, and respiration rate) will only be recorded on the first day of radiation therapy (C1D8).
15. Complete physical exam to be completed by the investigator or qualified designee at screening, C1D1 and start of all subsequent cycles. Complete physical exam includes skin, head, eyes, throat, neck, joints, lungs, heart, abdomen (including liver and spleen), lymph nodes, extremities, and neurological exam. The exam may be performed within 3 days prior to the day of each scheduled study visit. For C1D15 only, a complete physical and neurological exam must be performed. For all subsequent mid-cycle visits, ie for all subsequent D15 and D29 visits, only a symptom-directed physical exam will be performed.
16. Symptom-direct physical exam to be completed as clinically indicated by the investigator or qualified designee at the first day of radiation therapy (C1D8), all mid-cycle visits (i.e. all Day 15 and 29 visits except the C1D15 visit), and post-study drug visits (30-day and 90-day visits)
17. Serum Chemistry includes: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase and lipase. Samples may be collected within 3 days prior to the scheduled day of each visit.
18. Hematology includes: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count. Samples may be collected within 3 days prior to each scheduled study visit.

19. Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein. Samples may be collected within 3 days prior to each scheduled study visit.
20. Urine or serum β-HCG is required for women of child-bearing potential at the indicated study timepoints.
21. TSH (thyroid stimulating hormone) and free T4 (free thyroxine) are require at the indicated study timepoints.
22. 12-lead ECG: A standard 12-lead ECG will be required only at screening.
23. Chest X-ray: Required only at screening if not performed within 60 days prior to initiation of study treatment.
24. Radiation therapy (RT): Administered 3 Gy per fraction for 15 fractions. Total RT dose will be 45 Gy. See Section 5.2.2 for technical details of RT. On the first day (C1D8) of HFRT, adverse event assessment, a symptom-directed physical exam and limited vital signs (temperature, resting blood pressure, pulse, and respiration rate) must be performed.
25. Nivolumab will be administered every 2 weeks (±3 days of scheduled visit) in an outpatient setting as an IV infusion. The nivolumab dose will depend on individual body weight. The dose of nivolumab must be adjusted each dose for changes in body weight of ≥10%. Dose adjustments for changes in body weight of <10% will be at the discretion of the investigator. Nivolumab administration will continue for all subjects for 2 years (96 weeks) or until disease progression, intolerable toxicity, death, or the subject meets another withdrawal criterion per protocol.
26. Ipilimumab will be administered every 6 weeks (±5 days of scheduled visit) in an outpatient setting as an IV infusion. The ipilimumab dose will depend on individual body weight. The dose of ipilimumab must be adjusted each dose for changes in body weight of ≥10%. Dose adjustments for changes in body weight of <10% will be at the discretion of the investigator. Ipilimumab administration will continue for all subjects for 2 years (96 weeks) or until disease progression, intolerable toxicity, death, or the subject meets another withdrawal criterion per protocol.
27. Contrast-enhanced brain MRI: A contrast-enhanced MRI must be obtained within 21 days of the first dose of study treatment. For response assessment, a contrast-enhanced brain MRI will be performed 8 weeks after study drug initiation, and then every 8 weeks thereafter. MRIs can be performed within 7 days (±7 days) of scheduled assessment. On-study imaging should follow calendar days (every 8 weeks) and should not be adjusted for delays in cycle starts. Local reading (investigator assessment) will be used to determine eligibility and for participant management. Response Assessments will be performed on every brain imaging assessment performed on protocol per RANO criteria, although non-traditional iRANO criteria may be used for patient management (see **Appendix 3**).
28. Research Blood Samples: For <u>all</u> patients, whole blood will be drawn at two timepoints: At pre-dose C1D1 (-3 days) and at C2D1(± 3 days). Four purple top EDTA tubes (40 mL) will be drawn, one EDTA tube will be sent to the CBRD and three EDTA tubes will be sent immediately to the IMC.
29. Research (sodium) MRI: All subjects must have a research MRI performed prior to C1D1 (within 21 days of study drug initiation) and at every response assessment timepoint on study treatment (i.e., every 8 weeks after study drug initiation, and then every 8 weeks thereafter). Research MRIs can be performed within 7 days (±7 days) of scheduled assessment. Research MRIs must be performed at NYUSoM.
30. <u>Optional</u> tumor biopsy: If subjects provided consent for optional on-study or post-study drug tumor biopsy collection, a tumor specimen will be collected for research purposes. For details see the study procedures.
31. End of treatment assessments will be performed within 7 days after decision to end treatment. If the date to end treatment is determined later than 14 days from last study drug treatment, then it can be combined with the 30-day visit. All participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 30 days of last study drug.
32. A site visit is to be performed at 30 days (±7 days) after the last study drug is given, unless the subject is unable to travel due to deteriorating medical condition. The visit will include the indicated safety procedures.
33. A site visit is to be performed at 90 days (±14 days) after the last study drug is given, unless the subject is unable to travel due to deteriorating medical condition. The visit will include the safety procedures detailed below, with the exception of serum chemistry, hematology, TSH, and free T4, which are required only if study-related toxicity persists.
34. Survival follow up: Following the 90-day post-drug visit, all subjects will be contacted every 3 months (±14 days) to assess for study-treatment related toxicities, initiation of any new anti-cancer treatments, and survival status. Contact may be performed by a site visit, telephone contact, e-mail, or mail. The date of death, initiation of any new anti-cancer treatments and date of last contact should be documented if this information is available. All subjects will be followed post-treatment until death, withdrawal of permission to record at least survival data, or subject is lost to follow-up. If a subject withdraws permission to record at least survival data after coming off treatment, this must be documented along with the date the subject withdraws permission as per details in Section 6.12. All reasonable efforts must be made to locate subjects to determine and report their ongoing

status. Lost to follow-up is defined by the inability to reach the subject (or the subject's next-of-kin if the subject does not respond) after a minimum of two documented phone calls, faxes, or emails in a 3 month period over two consecutive 3 month periods, a lack of response by subject to one registered mail letter, and if no medical records are available to be reviewed (See study procedures for details).

35. T cell subsets (CD3, CD4, and CD8) will be collected at the following 5 timepoints only: baseline (pre-C1D1), C2D1, C4D1, C6D1, and C8D1.
36. After informed consent, if subjects undergo tumor resections or biopsies prior to treatment initiation, during the study period, or after progression on study drug, a small amount fresh surgical specimen (approximately 0.5 cm2, approximately equivalent to amount required for a stereotactic needle biopsy) will be collected for short-term (6-8 days) patient-derived organotypic tumor spheroid (PDOTS) culture and for dissociation into single cell suspensions for flow-cytometry-based immunoprofiling. Tumor sample will be collected in medium (DMEM) in a sterile cryotube and immediately transported on ice to Dr. Kwok-Kin Wong's laboratory at NYU Langone for immediate processing.

15.2 Appendix 2: Schedule of Events for Surgical Study Cohort

STUDY DAY ►	Day -50 to -29 [2]	C1 D-29[12]	C1 D-15	C1 D1	C1 D8[16]	C1 D15	C1 D29 and C2 D1	Week 8+ [27]	End of Rx[31]	30-Day PostDrug[32]	90-Day PostDrug [33]	Surv F/u[34]
Study Team Procedures												
Informed Consent [1]	X											
Demographics	X											
Inclusion/Exclusion Criteria [3]	X											
Medical/Disease History [4]	X											
MGMT testing [6]	X											
KPS Assessment [9]	X	X	X	X			X		X	X	X	
Concomitant Medications [10]	X	X	X	X		X	X		X	X	X	
Height and Weight [13]	X	X	X	X		X	X		X	X	X	
Vital Signs [14]	X	X	X	X	X	X	X		X	X	X	
Complete Physical Exam [15]	X	X		X		X[15]	X		X			
Directed Physical Exam [16]			X		X	X[16]				X	X	
Adverse Events [11]	X	X	X	X	X	X	X		X	X	X	
Survival									X	X	X	X
Laboratory/Cardiology Assessments												
Serum Chemistry [17]	X	X	X	X		X	X		X	X	X[33]	
Hematology [18]	X	X	X	X		X	X		X	X	X[33]	
T cells (CD3, CD4, CD8) [35]	X		X	X		X[35]						
Urinalysis [19]	X		X	X		X	X		X			
Coagulation [7]	X		X	X								
Hepatitis B and C [8]	X											
Pregnancy Test [20]	X	X				X			X	X	X	
TSH, free T4 [21]	X	X				X			X[19]	X	X[33]	
12-Lead ECG [22]	X											
Study Treatment												
Radiotherapy: 3 Gy x 15 fractions (45 Gy total) [24]					X	X	X					
Nivolumab IV [25]		X		X		X	X					

Ipilimumab IV [26]		X				X						
Imaging Assessments												
MRI Brain [27]	X		X[27]					X[27]				
Chest X-ray [23]	X											
Research Procedures												
Archival tumor tissue [5]	X											
Research blood samples [28]		X	X			X						
Research MRI (sodium MRI) [29]	X							X[29]				
Surgical tumor specimen [36]	X[36]		X[36]									
Tumor biopsy (optional) [30]									X			

One treatment cycle will be defined as 42 days

EXPLANATION OF SUPERSCRIPTS:

1. Informed Consent: Performed by MD attending only. Informed consent process to be fully document: e.g. prospective participant had sufficient time for deliberation, all questions were answered, treatment options provided by MD, full study reviewed including risks, and a copy of signed consent given to the participant. No study specific screening procedures may occur until after the informed consent process is complete. Informed consent may be obtained more than 21 days before the start of screening procedures. Assessments performed as part of standard of care that fall within the screening window but before informed consent is obtained may be used for screening, and need not be repeated for enrollment eligibility.
2. Screening will start within 21 days prior to initiation of study treatment (first dose of study drug).
3. Inclusion/exclusion criteria: source documentation providing investigator's confirmation that the participant had met all eligibility criteria must be available prior to registration.
4. All patients must have histopathological documentation of glioblastoma or gliosarcoma (WHO grade IV). Subjects must not have had any prior anti-tumor therapy for glioblastoma or gliosarcoma other than surgery. Medical history should also include review of any ongoing medical conditions and medical history pertaining to eligibility on study and involvement during study.
5. All patients will be required to provide a tumor block or 20 unstained slides from a tissue specimen that demonstrates glioblastoma or gliosarcoma (WHO grade IV). See study procedures for details.
6. *MGMT* testing: *MGMT* promoter methylation status must be determined by central testing at NYUSoM. Only subjects with unmethylated MGMT promoter confirmed by central testing are eligible. See study procedures for details of *MGMT* testing.
7. Coagulation – PT/INR, PT, PTT required at screening only.
8. Hepatitis B virus surface antibody, hepatitis B virus surface antigen, hepatitis B virus core antibody, and hepatitis C virus antibody at screening only.
9. Karnofsky Performance Status (KPS) assessment: See Appendix 2 for KPS scale.
10. Concomitant medication recording will be ongoing throughout the course of the study. Record concomitant medications from within 21 days before starting study treatment up to the 90-Day Post Drug Visit.
11. All adverse events must be monitored throughout the study via safety assessments, observation, and participant reporting and for 100 days after the last dose of nivolumab and 90 days after the last dose of ipilimumab.
12. Baseline (Pre-C1D1) assessments must be performed within 3 days prior to the first dose of study drug. Evaluations performed at screening that fall within 3 days of treatment initiation will not need to be repeated. The baseline labs do not need to re-meet the specific eligibility criteria, only meet criteria for dosing
13. Height is required only at screening. Weight can be taken within 3 days of scheduled study drug administration.

14. Vital signs include temperature, resting blood pressure, pulse, and respiration rate. When scheduled at the same visit as other procedures, vital signs should be measured prior to clinical laboratory assessments or research blood sample collection. At C1D1, vital signs will be collected prior to treatment, at the end of the infusion, and every 30 minutes for the first hour post-infusion (all VS timepoints ±10 minutes). Vital signs on subsequent treatment days of cycle 1 and all subsequent cycles will be assessed and documented prior to the infusion, and then 30 minutes (±10 minutes) after the completion of the infusion. During Radiation Therapy, limited vital signs (temperature, resting blood pressure, pulse, and respiration rate) will only be recorded on the first day of radiation therapy (C1D8).
15. Complete physical exam to be completed by the investigator or qualified designee at screening, C1D1 and start of all subsequent cycles. Complete physical exam includes skin, head, eyes, throat, neck, joints, lungs, heart, abdomen (including liver and spleen), lymph nodes, extremities, and neurological exam. The exam may be performed within 3 days prior to the day of each scheduled study visit. For C1D15 only, a complete physical and neurological exam must be performed. For all subsequent mid-cycle visits, ie for all subsequent D15 and D29 visits, only a symptom-directed physical exam will be performed.
16. Symptom-direct physical exam to be completed as clinically indicated by the investigator or qualified designee at the first day of radiation therapy (C1D8), all mid-cycle visits (i.e. all Day 15 and 29 visits except the C1D15 visit), and post-study drug visits (30-day and 90-day visits)
17. Serum Chemistry includes: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, glucose, albumin, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), amylase and lipase. Samples may be collected within 3 days prior to the scheduled day of each visit.
18. Hematology includes: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count. Samples may be collected within 3 days prior to each scheduled study visit.
19. Urinalysis: glucose, blood, pH, specific gravity, ketones, and urine protein. Samples may be collected within 3 days prior to each scheduled study visit.
20. Urine or serum β-HCG is required for women of child-bearing potential at the indicated study timepoints.
21. TSH (thyroid stimulating hormone) and free T4 (free thyroxine) are require at the indicated study timepoints.
22. 12-lead ECG: A standard 12-lead ECG will be required only at screening.
23. Chest X-ray: Required only at screening if not performed within 60 days prior to initiation of study treatment.
24. Radiation therapy (RT): Administered 3 Gy per fraction for 15 fractions. Total RT dose will be 45 Gy. See Section 5.2.2 for technical details of RT. On the first day (C1D8) of HFRT, adverse event assessment, a symptom-directed physical exam and limited vital signs (temperature, resting blood pressure, pulse, and respiration rate) must be performed.
25. Nivolumab will be administered every 2 weeks (±3 days of scheduled visit) in an outpatient setting as an IV infusion. The nivolumab dose will depend on individual body weight. The dose of nivolumab must be adjusted each dose for changes in body weight of ≥10%. Dose adjustments for changes in body weight of <10% will be at the discretion of the investigator. Nivolumab administration will continue for all subjects for 2 years (96 weeks) or until disease progression, intolerable toxicity, death, or the subject meets another withdrawal criterion per protocol.
26. Ipilimumab will be administered every 6 weeks (±5 days of scheduled visit) in an outpatient setting as an IV infusion. The ipilimumab dose will depend on individual body weight. The dose of ipilimumab must be adjusted each dose for changes in body weight of ≥10%. Dose adjustments for changes in body weight of <10% will be at the discretion of the investigator. Ipilimumab administration will continue for all subjects for 2 years (96 weeks) or until disease progression, intolerable toxicity, death, or the subject meets another withdrawal criterion per protocol.
27. Contrast-enhanced brain MRI: A contrast-enhanced MRI must be obtained within 21 days of the first dose of study treatment. For the Surgical Study Cohort, a post-operative MRI brain must be obtained within 72 hours from craniotomy and tumor re-resection on C1D-15. For response assessment, a contrast-enhanced brain MRI will be performed 8 weeks after study drug initiation, and then every 8 weeks thereafter. MRIs can be performed within 7 days (±7 days) of scheduled assessment. On-study imaging should follow calendar days (every 8 weeks) and should not be adjusted for delays in cycle starts. Local reading (investigator assessment) will be used to determine eligibility and for participant management. Response Assessments will be performed on every brain imaging assessment performed on protocol per RANO criteria, although non-traditional iRANO criteria may be used for patient management (see **Appendix 3**).

28. Research Blood Samples: For all patients, whole blood will be drawn at two time points: At pre-dose C1D-29 (-3 days) and at C2D15(±/- 3 days). Four purple top EDTA tubes (40 mL) will be drawn, one EDTA tube will be sent to the CBRD and three EDTA tubes will be sent immediately to the IMC.
29. Research (sodium) MRI: All subjects must have a research MRI performed prior to C1D1 (within 7 days of study drug initiation) and at every response assessment time point on study treatment (i.e., every 8 weeks after study drug initiation, and then every 8 weeks thereafter). Research MRIs can be performed within 7 days (±7 days) of scheduled assessment. Research MRIs must be performed at NYUSoM.
30. Optional tumor biopsy: If subjects provided consent for optional on-study or post-study drug tumor biopsy collection, a tumor specimen will be collected for research purposes. For details see the study procedures.
31. End of treatment assessments will be performed within 7 days after decision to end treatment. If the date to end treatment is determined later than 14 days from last study drug treatment, then it can be combined with the 30-day visit. All participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 30 days of last study drug.
32. A site visit is to be performed at 30 days (±7 days) after the last study drug is given, unless the subject is unable to travel due to deteriorating medical condition. The visit will include the indicated safety procedures.
33. A site visit is to be performed at 90 days (±14 days) after the last study drug is given, unless the subject is unable to travel due to deteriorating medical condition. The visit will include the safety procedures detailed below, with the exception of serum chemistry, hematology, TSH, and free T4, which are required only if study-related toxicity persists.
34. Survival follow up: Following the 90-day post-drug visit, all subjects will be contacted every 3 months (±14 days) to assess for study-treatment related toxicities, initiation of any new anti-cancer treatments, and survival status. Contact may be performed by a site visit, telephone contact, e-mail, or mail. The date of death, initiation of any new anti-cancer treatments and date of last contact should be documented if this information is available. All subjects will be followed post-treatment until death, withdrawal of permission to record at least survival data, or subject is lost to follow-up. If a subject withdraws permission to record at least survival data after coming off treatment, this must be documented along with the date the subject withdraws permission as per details in

    Section 6.12. All reasonable efforts must be made to locate subjects to determine and report their ongoing status. Lost to follow-up is defined by the inability to reach the subject (or the subject's next-of-kin if the subject does not respond) after a minimum of two documented phone calls, faxes, or emails in a 3 month period over two consecutive 3 month periods, a lack of response by subject to one registered mail letter, and if no medical records are available to be reviewed (See study procedures for details).
35. T cell subsets (CD3, CD4, and CD8) will be collected at the following 5 time points only: baseline (pre-C1D1), C2D1, C4D1, C6D1, and C8D1.
36. After informed consent, if subjects undergo tumor resections or biopsies prior to treatment initiation, during the study period, or after progression on study drug, a small amount fresh surgical specimen (approximately 0.5 cm2, approximately equivalent to amount required for a stereotactic needle biopsy) will be collected for shortterm (6-8 days) patient-derived organotypic tumor spheroid (PDOTS) culture and for dissociation into single cell suspensions for flow-cytometry-based immunoprofiling. Tumor sample will be collected in medium (DMEM) in a sterile cryotube and immediately transported on ice to Dr. Kwok-Kin Wong's laboratory at NYU Langone for immediate processing.

15.3 Appendix 3: Karnofsky Performance Status Scale

Karnofsky Performance Scale	
Percent	**Description**
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

15.4 Appendix 4: iRANO (immunotherapy response assessment in neuro-oncology) Criteria

Tumor response will be assessed every 8 weeks for patients treated on this study using RANO criteria, however non-traditional iRANO criteria[73,85] may be considered for patient management as outlined in the **Study Procedures** (**Section 6**).

Anti-Tumor Effect Definitions

Evaluable for objective response. Only those participants who have measurable disease present at baseline (obtained within 14 days of cycle 1, day 1) scan and have received at least one dose of therapy will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression or die prior to the end of cycle 1 will also be considered evaluable.)

Measurable disease. Bi-dimensionally, contrast-enhancing, measurable lesions with clearly defined margins by CT or MRI scan, with a minimal diameter of 1 cm, and visible on 2 axial slices which are at least 5 mm apart with 0 mm skip. Measurement of tumor around a cyst or surgical cavity, if necessary, requires a minimum thickness of 3 mm. If there are too many measurable lesions to measure at each evaluation, the investigator must choose the largest two to be followed before a participant is entered on study. The remaining lesions will be considered non-measureable for the purpose of objective response determination. Unless progression is observed, objective response can only be determined when all measurable and non-measurable lesions are assessed.

Non-measurable evaluable disease. Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with maximal diameter < 1cm.

Response/Progression Categories

Complete response (CR). All of the following criteria must be met:

1. Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.
2. No new lesions*.
3. All measurable and non-measurable lesions must be assessed using the same techniques as baseline.
4. Participants must be on no steroids or must not be on increased doses of steroids within 2 weeks of assessment relative to the dose taken at the time of the previous assessment
5. Stable or improved non-enhancing (T2/FLAIR) lesions
6. Stable or improved clinically, for clinical signs and symptoms present at baseline and recorded to be disease related

Participants with non-measurable disease cannot have a complete response. The best response possible is stable disease.

**See immunotherapy considerations below regarding new lesions*

Partial response (PR). All of the following criteria must be met:

1. Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.
2. No progression of non-measurable disease.
3. No new lesions.*

4. All measurable and non-measurable lesions must be assessed using the same techniques as baseline.
5. Participants must be on no steroids or must not be on increased doses of steroids within 2 weeks of assessment relative to the dose taken at the time of the previous assessment
6. Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.
7. Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

Participants with non-measurable disease cannot have a partial response. The best response possible is stable disease.

**See immunotherapy considerations below regarding new lesions*

Progressive disease (PD). Any of the following criterion must be met:

1. 25% increase in sum of the products of perpendicular diameters of enhancing lesions (over best response or baseline if no decrease) on stable or increasing doses of corticosteroids
2. Patients who decrease corticosteroid use within 2 weeks of MRI assessment relative to the dose taken at the time of the previous assessment cannot be classified as having progressive disease and should be classified as non-evaluable.
3. Any new enhancing measurable lesion*
4. Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication side effects, complications of therapy, cerebrovascular events, infection, etc.). The definition of clinical deterioration is left to the discretion of the investigator but it is recommended that a decline in the Karnofsky Performance Score (KPS) from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration, unless attributable to co-morbid events or changes in corticosteroid dose.
5. Failure to return for evaluation due to death or deteriorating condition

**See immunotherapy considerations below regarding new lesions*

Stable disease (SD). All of the following criteria must be met:

1. Does not qualify for CR, PR, or progression.
2. All measurable and non-measurable sites must be assessed using the same techniques as baseline.
3. Stable clinically.

Unknown response status. Progressive disease has not been documented and one or more measurable or non-measurable lesions have not been assessed.

Special Considerations for Immunotherapies.

Appearance of new lesions is a criterion that defines progression of disease by RANO criteria. However, transient appearance of new enhancing lesions at either local or distant sites might occur in patients with neuro-oncological malignancies receiving immunotherapy. In such situations, careful radiological and clinical assessments are warranted. In some cases, new enhancing lesions might represent immune responses directed against infiltrative brain tumor cells. In addition, Immune-related response criteria guidelines for non-brain tumor cancers state that early increases in lesion size or new lesions do not define progressive disease unless further progressive changes are confirmed upon follow-up imaging, provided that patients do not have a clinical decline.[74] Therefore, confirmation to define progressive disease is an important, novel aspect of immune-related response criteria. Additionally, the converse argument, the need of follow-up imaging to confirm a radiographic response, has been an accepted component of most response assessment metrics including RANO.

Confirmation of radiographic progression to define progressive disease in iRANO:

- iRANO recommends confirmation of disease progression on follow-up imaging **3 months after initial radiographic progression** if there is no new or substantially worsened neurological deficits that are not due to comorbid events or concurrent medication, and it is 6 months or less from starting immunotherapy.
- Imaging within the 3-month follow-up can be done as medically appropriate at the discretion of the treating clinician.
- The appearance of new lesions 6 months or less from the initiation of immunotherapy alone does not define progressive disease.
- If follow-up imaging confirms disease progression, the date of actual progression should be back-dated to the date of initial radiographic progression.

Note on continuation of therapy pending confirmation of radiographic disease progression: **iRANO** recommends confirmation of disease progression on **follow-up imaging 3 months after initial radiographic progression** if there is no new or substantially worsened neurological deficits that are not due to comorbid events or concurrent medication, and it is 6 months or less from starting immunotherapy (see **Appendix 3** for **iRANO** criteria details). A decision of whether a patient should continue immunotherapy pending confirmation of radiographic disease progression should be established based on perceived benefits and risks. Continuation of immunotherapy may be considered pending follow-up imaging as long as subjects are deriving apparent clinical benefit with minimal and acceptable toxic effects.

By contrast, investigators may consider interrupting immunotherapy for subjects who need a substantial increase in corticosteroids (i.e., >4 mg of dexamethasone or equivalent per day) for evolving symptoms associated with brain edema or who have more than mild treatment-related toxic effects such as at least grade 2 irAEs. These guidelines are included to limit the likelihood of progressive immunotherapy-induced inflammatory changes leading to substantial deficits in otherwise stable or symptom-free patients. In such subjects, an interruption of immunotherapy dosing might be considered pending follow-up imaging.

Furthermore, investigators may discontinue or interrupt immunotherapy at any time if this option seems to be in the best medical interest of the subjects. As a general guidance, resumption of immunotherapy might be taken into account when systemic dexamethasone is decreased to 4 mg/day or less and the contrast-enhancing tumor burden is classified as stable disease, partial response, or complete response on a follow-up scan, or when relevant treatment-related toxic effects have resolved to grade 1 or less or pre-treatment baseline.

The iRANO Response Criteria to be used in this study are summarized in the Table below.

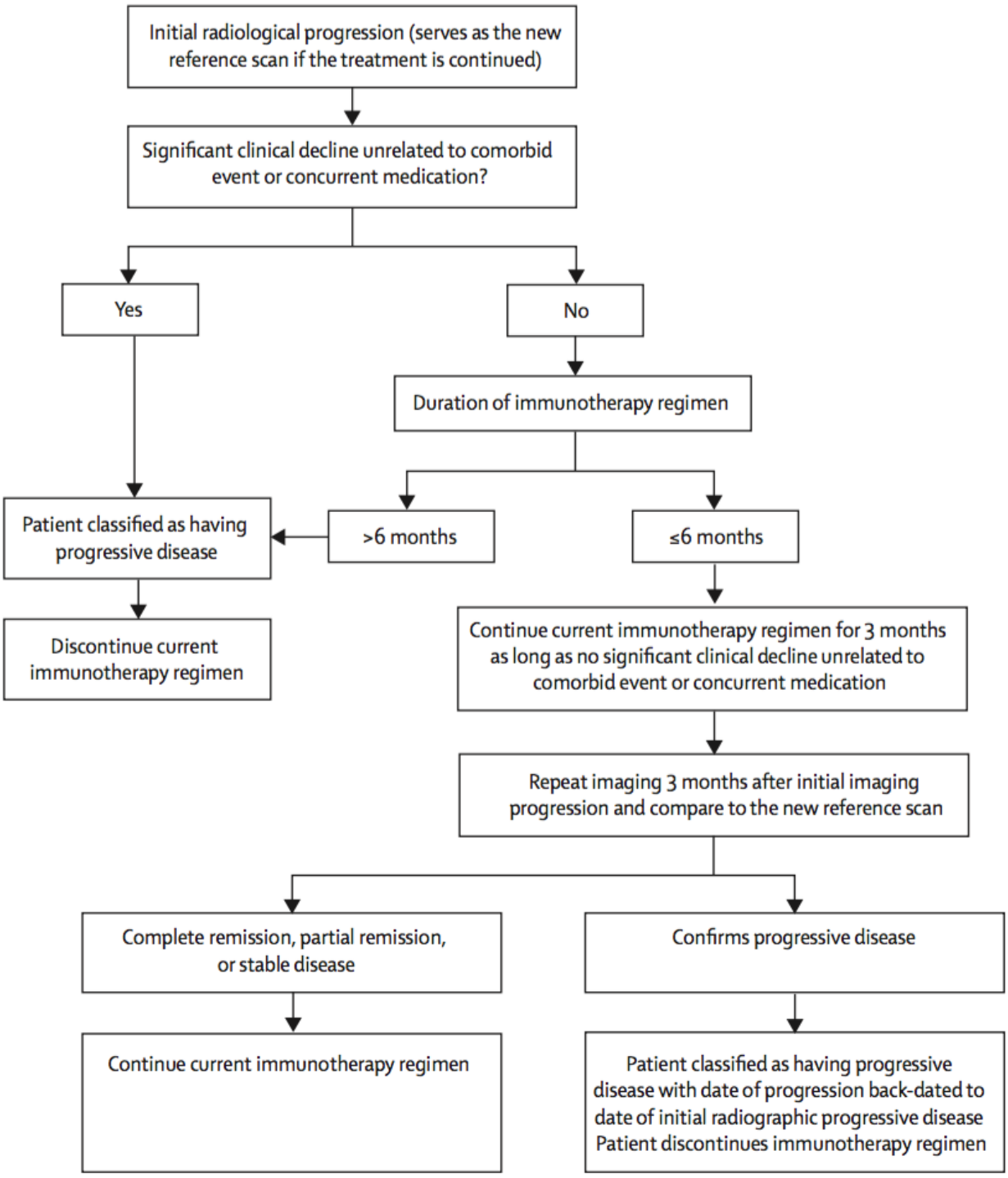
Summary of iRANO Response Criteria

Complete response	Disappearance of all enhancing disease for ≥4 weeks; no new lesions; stable or improved T2/FLAIR; no more than physiological steroids; clinically stable or improved
Partial response	≥50% decrease in the sum of biperpendicular diameters of enhancing disease for ≥4 weeks; no new lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved
Minor response	NA
Stable disease	Does not qualify for complete response, partial response, or progressive disease; no new lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved
Progressive disease	≥25% decrease in the sum of biperpendicular diameters of enhancing disease; or new lesions; or substantial worsened T2/FLAIR; or substantial clinical decline

Note: iRANO recommends confirmation of disease progression on follow-up imaging 3 months after initial radiographic progression if there is no new or substantially worsened neurological deficits that are not due to comorbid events or concurrent medication, and it is 6 months or less from starting immunotherapy. If follow-up imaging confirms disease progression, the date of actual progression should be back-dated to the date of initial radiographic progression. The appearance of new lesions 6 months or less from the initiation of immunotherapy alone does not define progressive disease. FLAIR=fluid-attenuated inversion recovery.

Note: Typographical error in the Progressive Disease definition, the correct criteria should be "increase" rather than "decrease".

iRANO treatment algorithm for the assessment of progressive imaging findings in patients with neuro-oncological malignancies undergoing immunotherapy



15.5 Appendix 5: Management Algorithms for Immuno-Oncology Agents

These general guidelines constitute guidance to the Investigator and may be supplemented by discussions with the Sponsor. The guidance applies to all immuno-oncology (I-O) agents and regimens, including nivolumab and ipilimumab.

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

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

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

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

The following pages include Management Algorithms to assist investigators in assessing and managing the following groups of AEs:

- Gastrointestinal
- Renal
- Pulmonary
- Hepatic
- Endocrinopathy
- Skin
- Neurological (non-brain edema related)

Note: For **Rules for managing brain/intracranial edema**, see Section 6.1.3.7.

For subjects who are suspected to have study treatment-related **symptomatic intracranial or brain edema** at any time during the study, **VEGF inhibition (bevacizumab) is preferred over corticosteroids** for management of suspected intracranial or brain edema, radiation necrosis, or intracranial hypertension, due to the risk that corticosteroids may suppress immune response against the tumor. See section 6.1.3.8 for details on VEGF inhibition in this study.

GI Adverse Event Management Algorithm

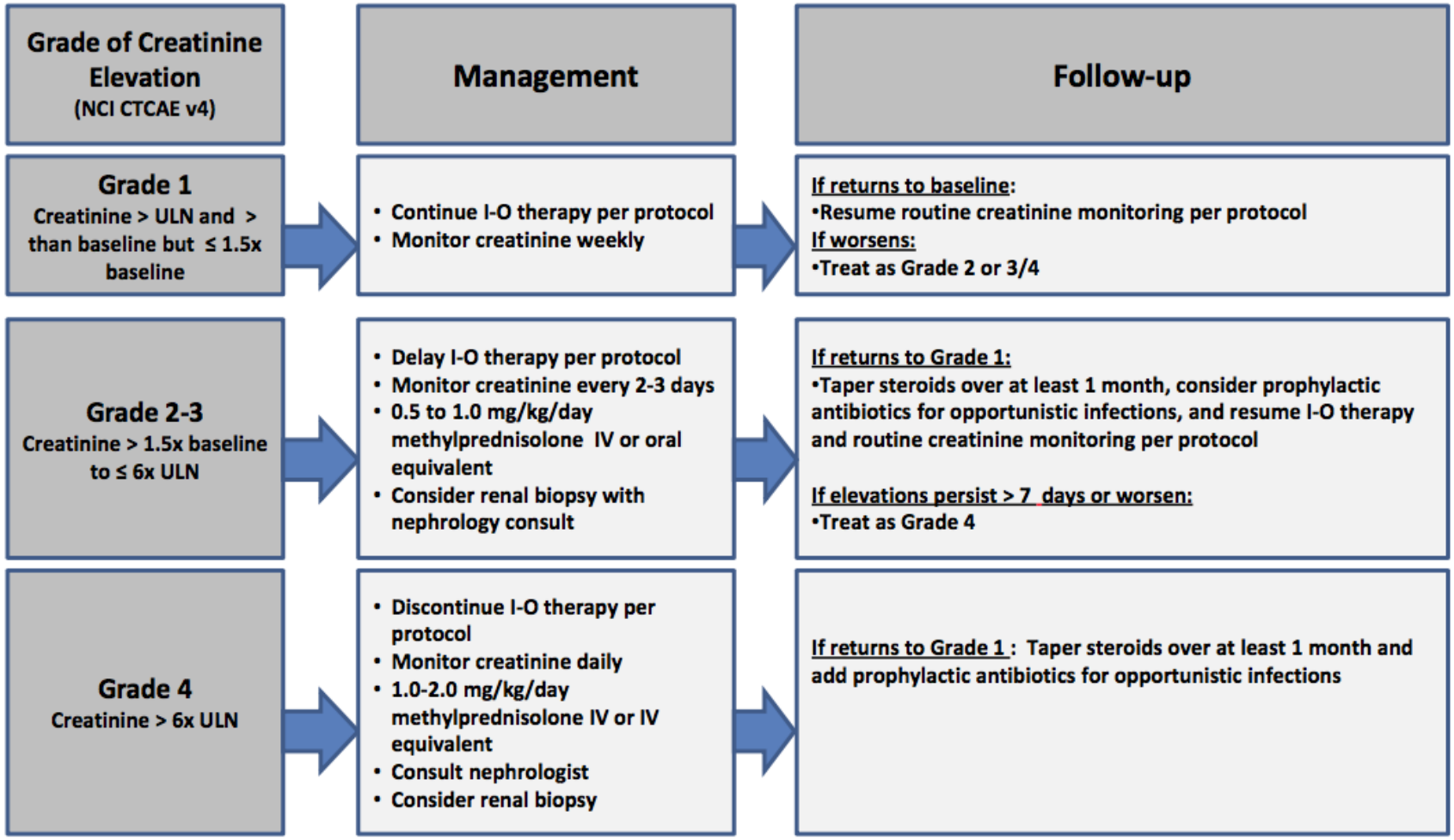
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

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

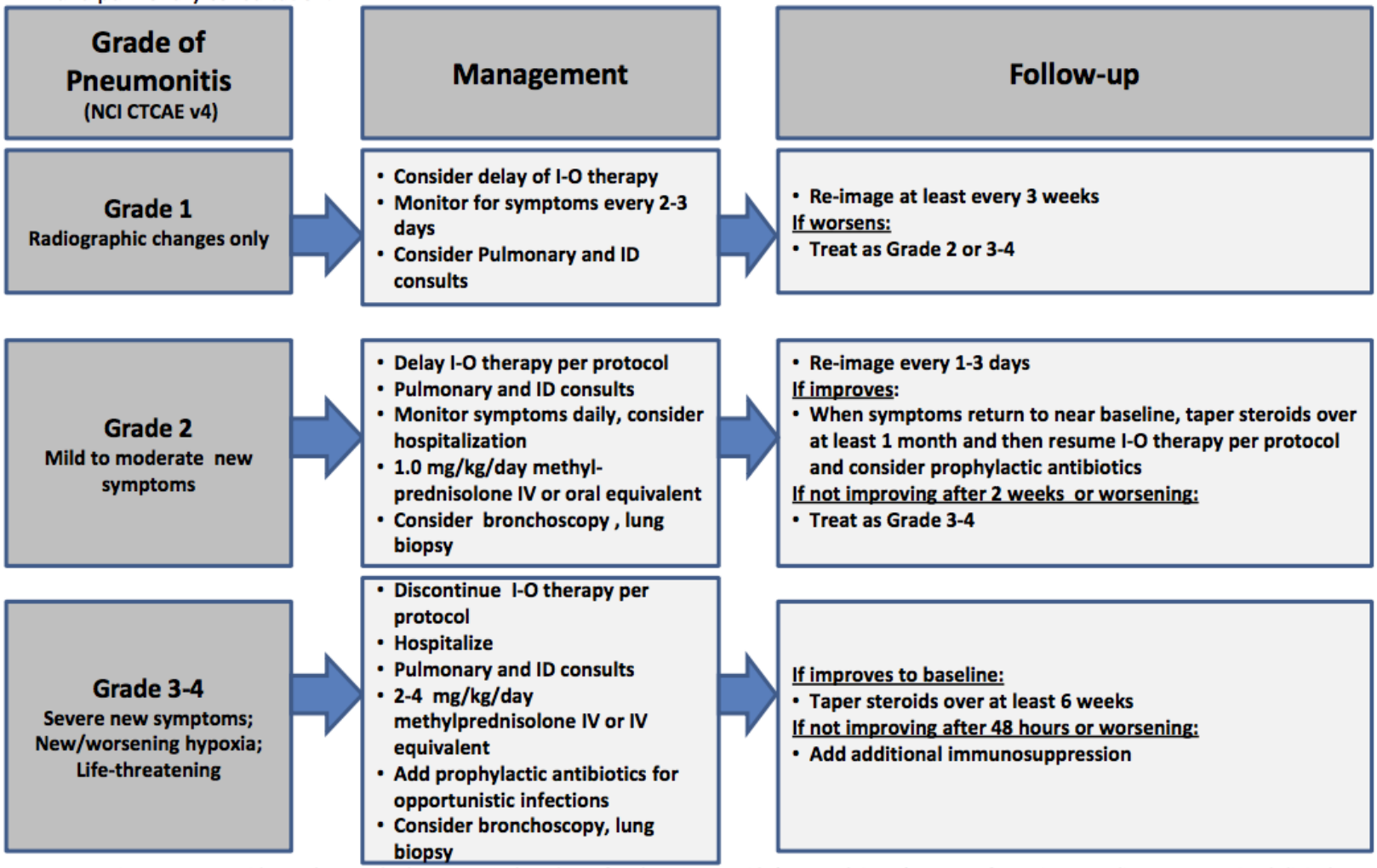
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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

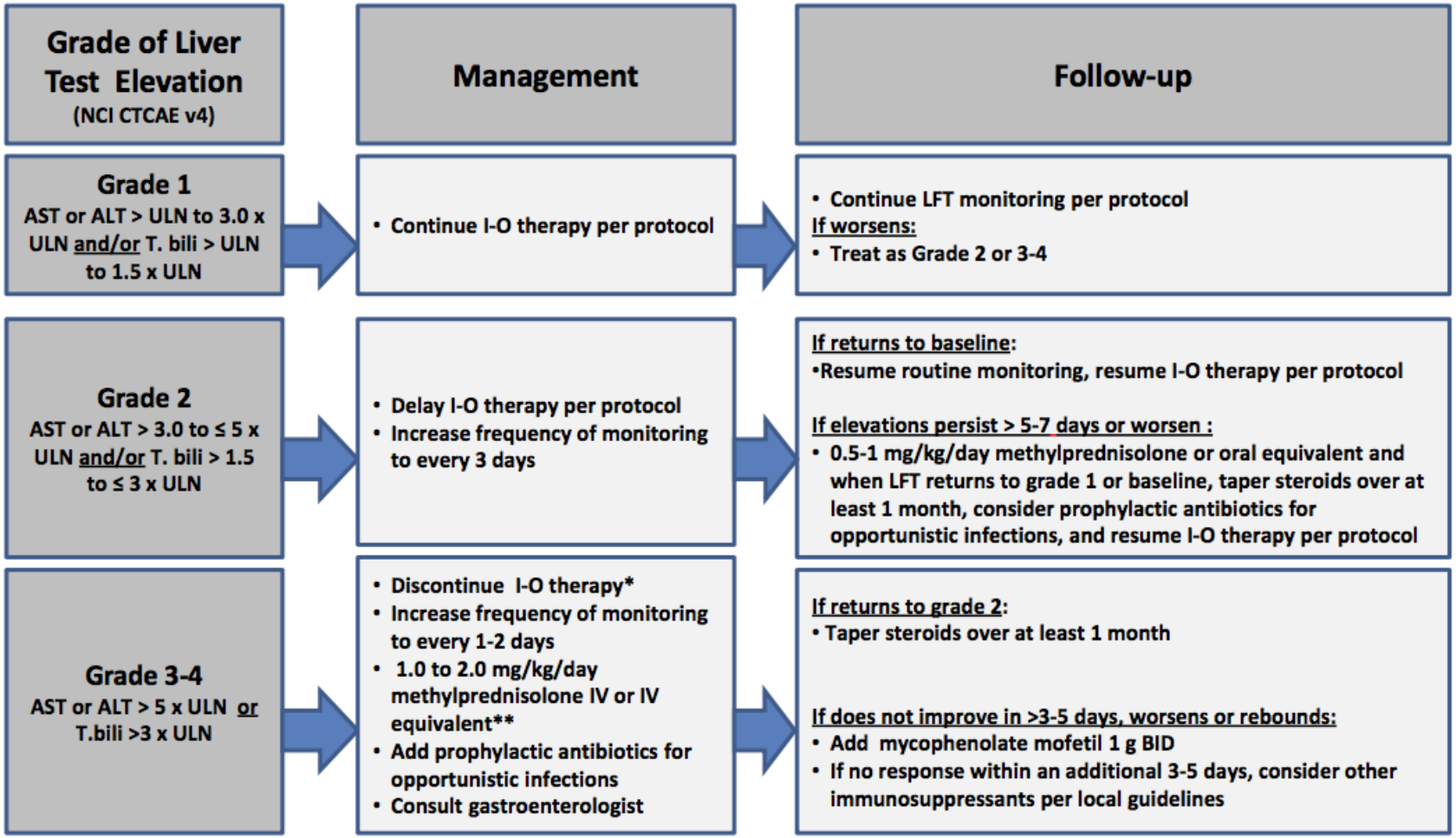
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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.



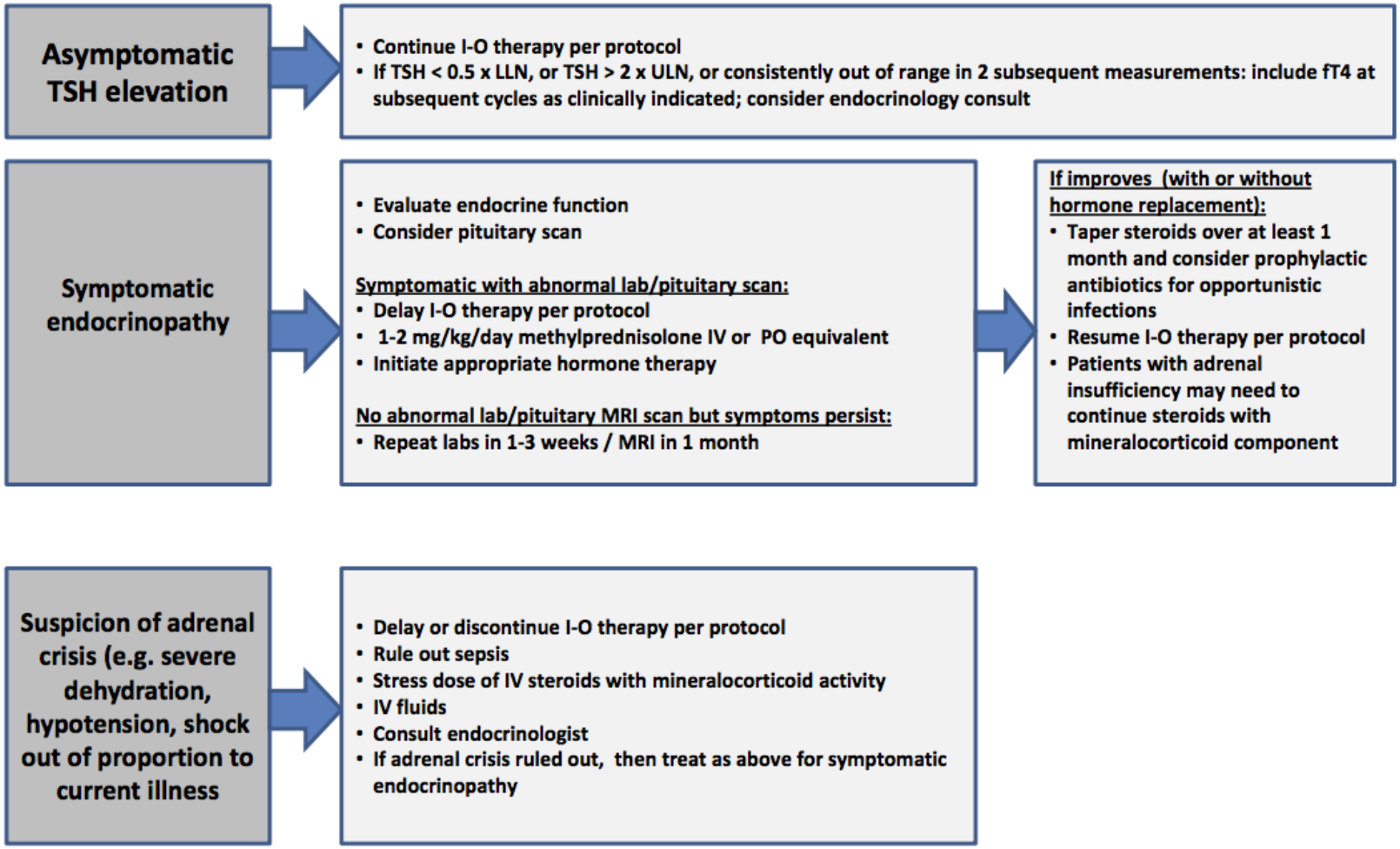
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

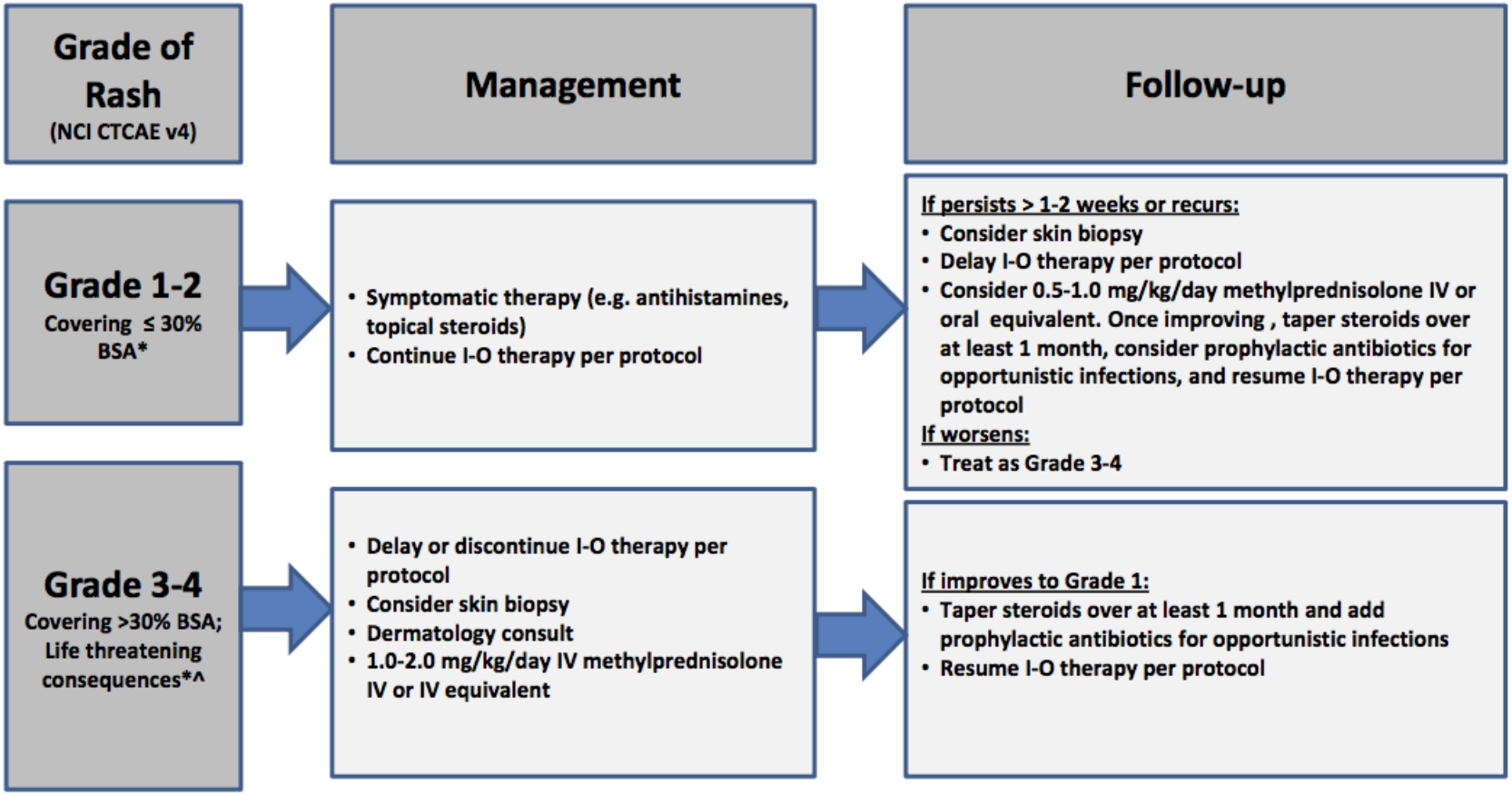
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

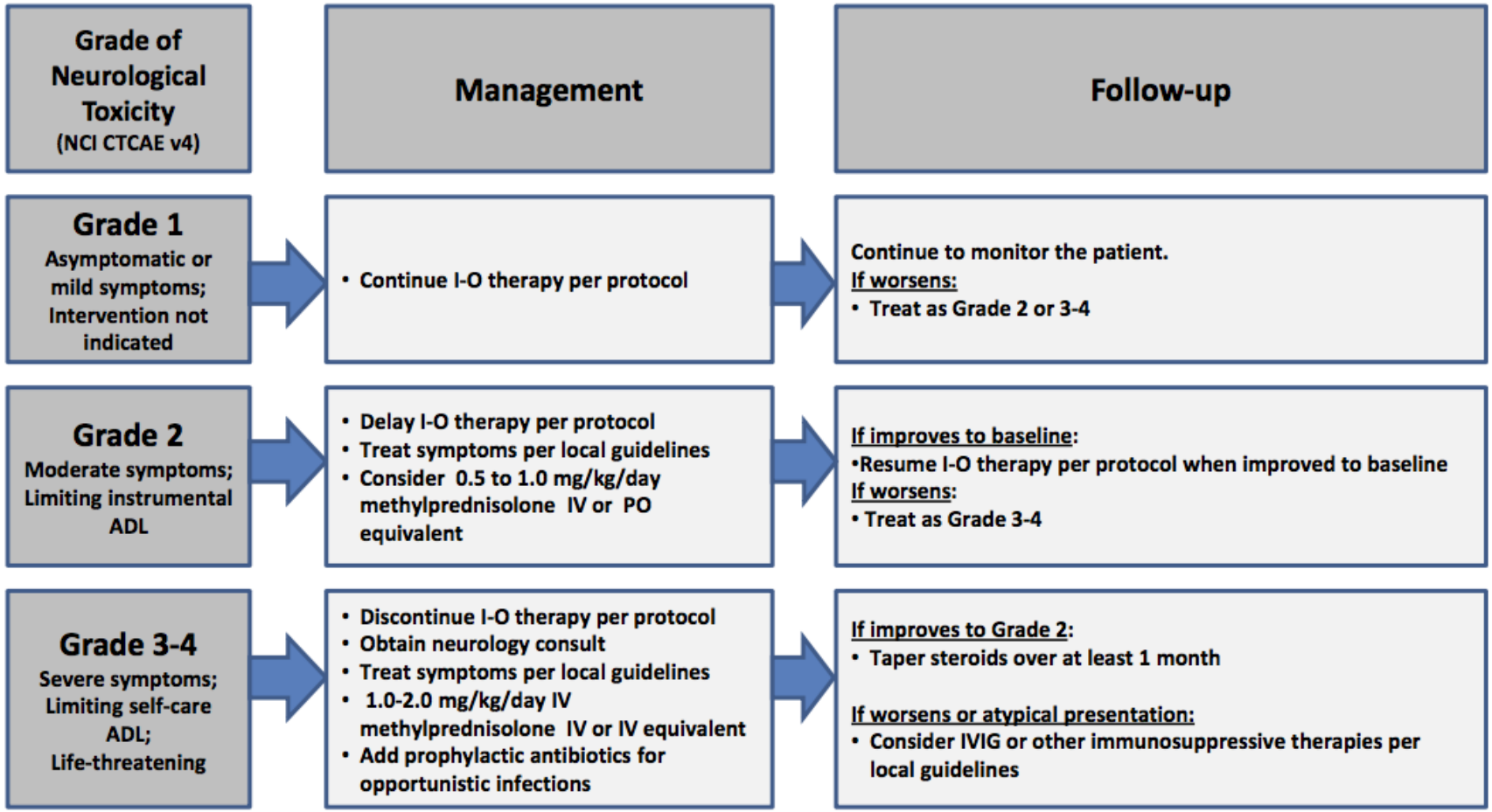
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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.

15.6 Appendix 6: Factors to Consider in Assessing the Relationship of AEs to Nivolumab, Ipilimumab or Infusion Procedure, Study Procedure, or Combination Treatment

Is there a reasonable possibility that the event may have been caused by the study drugs or infusion procedure, study procedure, or concomitant treatment?

No:

- due to external causes such as environmental factors or other treatment/s being administered
- due to the patient's/subject's disease state or clinical condition
- do not follow a reasonable temporal sequence following the time of administration of the dose of nivolumab or ipilimumab, study procedure, or combination treatment
- do not reappear or worsen when dosing with nivolumab or ipilimumab, study procedure, or combination treatment is resumed
- are not a known response to nivolumab or ipilimumab or infusion procedure, study procedure, or combination treatment based upon pre-clinical data or prior clinical data

Yes:

- could not be explained by environmental factors or other treatment/s being administered
- could not be explained by the patient's disease state or clinical condition
- follow a reasonable temporal sequence following the time of administration of the dose of nivolumab or ipilimumab
- resolve or improve after discontinuation of nivolumab or ipilimumab, study procedure, or combination treatment
- reappear or worsen when dosing with nivolumab or ipilimumab, study procedure, or combination treatment is resumed
- are known to be a response to nivolumab or ipilimumab or the infusion procedure, study procedure, or combination treatment based upon pre-clinical data or prior clinical data

NOTE: This list is not exhaustive.

15.7 Appendix 7: Research Biomarker/Correlative Studies: Background and Rationale

Note: This study involves collection of required archival tumor tissue and blood specimens for research as well as collection of an optional tumor specimen if a biopsy is required during or after protocol therapy. All patients will be given the option to have leftover research specimens after study completion and completion of protocol-defined research studies banked for future research at the time of informed consent. See **Section 9.1.1** for details on leftover research samples.

A. Research Specimens to be Collected

1. Archival tumor tissue

A paraffin-embedded or frozen tumor-tissue block with a minimum of 1 cm^2 of tumor surface area containing at least 20% viable tumor from a tissue specimen that demonstrates one of the two criteria specified below (also in Inclusion Criteria). If a tumor block cannot be submitted, then 20 unstained 5-micron slides from the tumor specimen must be submitted.

2. (Optional) Tumor Biopsy

If subjects undergo tumor resections or biopsies during the study period after treatment initiation, or after progression on study, a tumor specimen will be collected for research purposes. A section of frozen tumor or a FFPE block (surface area of 1 cm^2 containing at least 20% viable tumor from the tumor surgery is preferred. If a frozen tumor specimen or a tumor block cannot be provided, then 20 unstained 5-micron slides from the tumor block should be sent.

Send all research tumor specimens (archival and optional tumor biopsy) to:

Matija Snuderl, MD
Department of Pathology, NYU School of Medicine
560 First Avenue
Tisch Hospital HW 451
New York, NY 10016
Phone: 646-501-5281, Fax: 212-263-7916, Pager: 917-205-5543
Email: Matija.Snuderl@nyumc.org

3. Research Blood Samples

Research blood samples will be taken at only **2 timepoints:** Baseline (prior to Cycle 1, Day 1) and at Cycle 2, Day 1 (Week 6), as per the study procedures (Section 6) and the Schedule of Events, Appendix 1.

Research blood samples will be collected from all subjects as detailed below:

- 40 mL (four 10 mL purple top EDTA tubes) of whole blood will be drawn and immediately sent to the below sites:
  - One (1) 10 mL tube will be sent to the Center for Biospecimen Research and Development (CBRD) at NYUSoM for acquisition of whole blood, buffy coat, and plasma samples.
  - Three (3) 10 mL tubes will be sent to the Immune Monitoring Core (IMC) at NYUSoM and immediately processed for isolation of PBMCs.

All blood samples from non-NYUSoM sites should be sent to the CBRD at NYUSoM at the address below. Research blood specimens collected from NYUSoM subjects will be transported immediately to either the CBRD or the IMC (address below) as described above.

Center for Biospecimen Research and Development (CBRD)
NYU School of Medicine
Medical Science Building
550 First Avenue, Berg 3rd Fl., Rm. 381
New York, NY 10016
CBRDResearchRequest@nyumc.org
1 (646) 501-4268

The Immune Monitoring Core (IMC)
NYU School of Medicine
522 First Avenue
Medical Science Building, MSB 367
New York, NY 10016
Debra.Morrison@nyumc.org

4. Research MRI (sodium MRI)

Research MRIs (sodium) will be performed on all subjects at baseline (within 21 days of study drug initiation) and and at every response assessment timepoint on study treatment (i.e., every 8 weeks after study drug initiation, and then every 8 weeks thereafter). Research MRIs can be performed within 7 days (±7 days) of scheduled assessment. Research MRIs must be performed at NYUSoM. Research MRIs will be performed on MRI equipment housed at the same NYU facility where patients obtain clinical MRIs.

We will explore whether metabolic brain sodium (^{23}Na) MRI (sodium MRI) can distinguish between tumor progression from immunotherapy-related treatment effect, i.e. "pseudoprogression". Response assessment in immunotherapy remains a challenge and this problem is magnified in neuro-oncology, where standard response assessment criteria can be unreliable even for standard therapies. Abnormal contrast-enhancement on conventional brain MRIs can indicate either tumor progression or treatment-related cell death/necrosis (pseudoprogression). Cell death/necrosis results in elevated tissue sodium concentration on ^{23}Na (sodium) MRI, therefore sodium MRI has the potential to differentiate between treatment-related cell death/necrosis versus tumor progression and serve as a non-invasive pharmacodynamic biomarker in GBM patients being treated with radiotherapy and/or immunotherapy.

We will perform sodium MRI in all subjects, correlating imaging findings with conventional contrast-enhanced MRI and histopathology if available (for patients who require surgery during or after the study and provide optional tumor biopsy consent). Sodium MRI can be implemented in clinical MRI scanners and does not require intravenous contrast agents.

Background: Metabolic brain sodium (^{23}Na) MRI is based on the direct detection of endogenous sodium ions (Na^+) from salt in brain tissues, instead of water protons (^{1}H) as in standard MRI. Cells in healthy tissues maintain a large sodium concentration gradient between the intracellular and extracellular compartments, and any impairment of energy metabolism or insult to the cell membrane integrity leads to an increase of intracellular sodium concentration. Sodium concentrations are therefore very sensitive to changes in the metabolic state of tissues and integrity of cell membranes.

Cell damage induced by therapy can be characterized by loss of ion homeostasis (the steady-state maintenance of asymmetric ion concentrations inside and outside cells) in the abnormal cells that constitute the malignant tumor, through changes in their pH, membrane depolarization, and dysregulation of trans-membrane ion transporters (such as the Na^+/K^+ pump, or the Na^+/H^+ and Na^+/Ca^{2+} exchangers),

leading ultimately to cell death. As a consequence, large variations of Na^+ concentrations inside the cells are induced, which can be quantified with ^{23}Na MRI *in vivo*.

Measuring variations of both intracellular sodium concentration C_1 (due to loss of ion homeostasis) and extracellular volume fraction α_2 (due to cell death) with ^{23}Na MRI may provide new metabolic information on the early effects of therapy on brain tumors, as reported by members of our group (references below).

References:
Madelin, G et al. A method for estimating intracellular sodium concentration and extracellular volume fraction in brain in vivo using sodium magnetic resonance imaging. Scientific reports 4, 4763 (2014).

Madelin, G et al. Repeatability of quantitative sodium magnetic resonance imaging for estimating pseudo-intracellular sodium concentration and pseudo-extracellular volume fraction in brain at 3 T. PLoS One 10, e0118692 (2015).

Madelin G et al. Biomedical applications of sodium MRI in vivo. J Magn Reson Imaging 38, 511-529 (2013).

Sodium MRI Protocol: ^{23}Na MRI will be acquired on a PRISMA clinical scanner, with a house-made dual tuned $^{1}H/^{23}Na$ radiofrequency coil. The sodium protocol consists of two 3D whole-brain acquisitions of durations: 8 min (for whole sodium in the brain), and 12 min (with suppression of the sodium signal in CSF and in the extracellular compartment). Sodium image resolution is 5 mm isotropic. In addition, a standard MPRAGE (5-6 min) and/or FLAIR (5-6 min) are added for tumor detection and coregistartion with ^{23}Na data. Allow about 5 min for shimming at the beginning of the scanning session. Total duration is about 30-35 min. Sodium images reconstruction is performed offline in Matlab (2-3 min). Post-processing to create the 3D brain maps of C_1 and α_2 is also performed in Matlab.

Statistical considerations: Imaging features of tumor cell death and inflammation will be defined as increased contrast-enhancement on conventional MRI, and a statistically significant elevation of mean tissue sodium concentration (TSC) by at least 25% on ^{23}Na MRI.

B. Types of Planned Analyses on Research Specimens

I. Studies using Tumor Tissue Samples

- Whole-exome deep sequencing to estimate of the total mutation burden, total mutation-associated neoantigens, and T cell receptor (TCR) diversity.
- RNA-Seq to assess expression of predicted mutation-associated neoantigens.
- Immunohistochemistry (IHC) to assess intratumoral PD-L1 expression and other immune marker expression [e.g. lymphocyte activation gene 3 (LAG3), indoleamine-2,3-dioxygenase (IDO) and T-cell immunoglobulin and mucin domain 3 (TIM3)], and PD-1+ tumor infiltrating lymphocytes (TILs)

II. Studies Using Blood Samples

- Flow cytometry for quantification of T_{reg} and myeloid-derived suppressor cell (MDSC) levels
- Immune cell populations as profiled by high-parameter flow cytometry that simultaneously quantifies expression of proteins related to checkpoint inhibitors (PD1, CTLA4, TIM3, TIGIT, 41BB, CD150, LAG3, BTLA), differentiation status (CD45RA, CCR7, CD57, CD95, CD127), and other traits (CD25, IL12R, CXCR3, CXCR6).
- Genomic DNA for paired tumor sequencing
- TCR deep sequencing to determine peripheral clonal T-cell expansion and TCR diversity

- Circulating tumor DNA analysis

C. Specific Planned Analyses

1. Tumor Tissue Whole-Exome Deep Sequencing

Objectives: To assess the association between PFS6 and:

A) Tumor Mutation Burden;

B) Total predicted neoantigen load;

C) T cell receptor (TCR) diversity

A) Tumor Mutation Burden: Recently, higher nonsynonymous mutation burden and hypermutation phenotype have been associated with clinical benefit to immune checkpoint inhibitors, including PD-1 inhibitors.[86-89] We will compare the association of tumor mutation burden between tumors that achieve 1yr-OS and those that do not. We will analyze archival GBM tissue from all patients with whole-exome deep sequencing.

B) Total predicted neoantigen load: We will compare the potential mutation-associated neoantigen load between tumors that achieve 1yr-OS and those that do not. Using somatic exome data, we will assess the hypermutant cases for their immunogenic potential in the context of each patient's major histocompatibility complex (MHC) haplotype. To assess the potential for mutant peptide binding, somatic exome data combined with each individual patient's class I HLA haplotype will be applied to an epitope prediction algorithm,[90,91] which provides an estimate of the total number of mutation-associated neoantigens in each tumor.

C) T cell receptor (TCR) diversity: One strategy to reverse the suppression of tumor immune responses involves the use of radiation therapy (RT), which has been shown to augment anti-cancer immune responses and enhance the efficacy of immune therapies in systemic cancer preclinical models and patients. Preclinical studies examining combined radiotherapy and checkpoint inhibition indicate that each activate mostly non-redundant immune stimulating mechanisms and the major contribution of radiotherapy appears to be increasing T-cell receptor (TCR) diversity. RT induces major histocompatibility complex (MHC) class I presentation, increases antigen presentation, and increases cytotoxic T cell (CTL) recognition of irradiated cells[35,36] and enhances the diversity of the TCR repertoire of the expanded peripheral T cell clones.[37]

We will characterize the baseline pre-treatment intratumoral TCR diversity and use intratumoral TCR diversity data (e.g., the top 100 most frequent intratumoral TCR clonotypes) to examine TCR clonotype frequencies in pre-treatment (Baseline) and post-treatment (C2D1) blood samples. Using combined tumor tissue and pre-treatment (baseline) blood, we will generate whole-exome sequencing data and analyze frequencies/counts of TCR clonotypes to estimate the TCR diversity. The Shannon's diversity index (DI)[92] normalized to the number of reads ($DI= -\Sigma(pi\ln pi)/\ln n$, where n is the number of clones, pi is the clonal frequency of the ith clone, and sigma is summed from i = 1 to i = n) was calculated for each sample. This gives a value between 0 and 1, where 0 is monoclonal and 1 is an even distribution of different clones.

If Post-treatment tumor tissue is available: Additionally, if patients undergo tumor resections or biopsies during study therapy or after progression on study, we will whole-exome sequence multiple spatially distinct regions, if possible, of the progressive tumor to determine whether alterations in the clonal hypermutation distribution, TCR clonotype distribution or predicted neoantigens have occurred.

2. Tumor Tissue RNA-Seq

Objective: To use tumor tissue RNA-Seq data to evaluate the fraction of predicted mutation-associated neoantigens derived from somatic whole-exome sequencing data that is expressed and assess the association of PFS6 and number of expressed mutation-associated neoantigens.

Expression of predicted mutation-associated neoantigens: We will estimate the proportion of predicted mutation-associated neoantigens that are expressed by RNA-Seq and compare the expressed predicted mutation-associated neoantigen load between tumors that achieve PFS6 and those that do not. Total RNA will be extracted from FFPE tumor tissue using RNA extraction kits, treated to remove genomic DNA, quantified, and analyzed for integrity. RNA-Seq libraries will be prepared using standard protocols to purify poly-adenylated mRNA, generate double-stranded cDNA and ligate adapters and then submitted for next-generation sequencing

Clonality of mutation-associated expressed neoantigens: If enough tumor tissue is available to assess distinct regions of an individual tumor, we will perform RNA-Seq analyses on different regions of each tumor to assess the clonality of expressed neoantigens.

3. Tumor Tissue Immunohistochemistry (IHC)

Objectives: To assess the association of PFS6 and:

A) Tumor tissue PD-L1 expression,

B) baseline density and subtype of TIL populations.

A) Tumor cell programmed death-ligand 1 (PD-L1) expression: We will assess the association of 1yr-OS with baseline membranous and diffuse tumor cell PD-L1 expression. Tumor PD-L1 has been associated with response to PD-1 inhibitor therapy in studies of other solid cancers (see Rationale and Background) however the association of tumor tissue PD-L1 expression and response to anti-PD-1 or anti-PD-L1 therapy is unknown.

IHC staining of paraffin-embedded sections for PD-L1 will be performed on archived formalin-fixed paraffin-embedded archival tumor specimens as previously described.[93] The IHC assay for PD-L1 will incorporate an anti–PD-L1 rabbit monoclonal antibody (clone 28-8), which was developed on an automated platform by Dako North America. Consecutive sections will be stained for PD-L1 and a negative control reagent to control for nonspecific staining. A sample will be deemed PD-L1 positive for membranous staining if ≥5% of tumor cells, in a minimum of 100 evaluable tumor cells, have observable PD-L1–positive staining at any intensity. Specificity of the 28-8 antibody clone has been extensively validated previously.[93]

In addition, we will explore the association of T_{reg} ($CD4^+CD25^+FoxP3^+$) levels in tumor tissue and in circulation at baseline. Circulating T_{reg} levels will be quantified as below, and tumor tissues will be stained for CD4 and FoxP3 to assess infiltrating T_{regs}.

B) Tumor Infiltrating lymphocytes (TILs): We will assess the density and subtype of TILs in baseline pre-treatment FFPE tumor tissue by IHC using markers such as CD3, CD4, CD8, CD56, FoxP3, and PD-1.

If Post-treatment tumor tissue is available: Additionally, if patients undergo tumor resections or biopsies during study therapy or after progression on study, we will assess for changes in tumor tissue PD-L1 expression and TIL density and subtype after study treatment.

4. Tumor immunoprofiling using Patient-Derived Organotypic Tumor Spheroid (PDOTS) Culture and Flow Cytometry

Objectives: Fresh surgical tumor specimens, if available, will be used for:

A) Flow cytometry-based immunoprofiling of dissociated, single cell suspensions.

B) Characterization of baseline cytokine profiles in patient-derived organotypic tumor spheroid (PDOTS) short term culture system[92,93] and change in cytokine profiles after immune checkpoint blockade.

A) Flow cytometry-based immunoprofiling of dissociated, single cell suspensions: A small portion of freshly resected tumor tissue (0.5 cm^2, equivalent to 4 stereotactic needle biopsy specimens) placed in a sterile cryotube in media (DMEM) will be taken immediately on ice to Dr. Kwok-Kin Wong's lab at NYULH. In the lab, half the tumor specimen will be immediately processed into single cell suspensions for flow cytometry-based immunoprofiling in the Immune Core at NYU. The other half of the specimen will be used for objective B below.

B) Characterization of baseline cytokine profiles in patient-derived organotypic tumor spheroid (PDOTS) short term culture system[92,93] and change in cytokine profiles after immune checkpoint blockade: Half of the fresh surgical tumor specimen will be used immediately for short-term (6-8 days) *ex vivo* PDOTS culture. PDOTS cultures isolated from human tumors retain autologous lymphoid and myeloid cell populations, thus incorporating features of the tumor microenvironment, in short-term 3-dimensional microfluidic culture. [92,93] The PDOTS microfluidic culture system was developed by Kwok-Kin Wong, MD, PhD, and these experiments will be performed in his lab at NYULH. Cell suspensions from the fresh surgical specimens will be prepared and loaded into 3D microfluidic devices. After 3 days, PDOTS culture fresh medium +/- immune checkpoint blockers (anti-PD-L1 and anti-CTLA4 antibodies) will be added. After further 3-5 days of PDOTS culture, conditioned medium will be obtained for baseline and post-immune checkpoint blockade cytokine and chemokine profiling using Luminex arrays.

All fresh surgical tumor specimen collected will be used for these specific purposes only. No cell lines will be generated and true genetic testing will not be performed.

5. Peripheral Blood Flow Cytometry

Objectives: To assess the association of 1yr-OS and baseline level of T_{regs} or MDSCs within PBMCs or their change after study treatment initiation

Circulating T_{regs} and MDSCs in PBMCs: We will test whether the baseline level of circulating regulatory T cells (T_{regs}) or myeloid-derived suppressor cells (MDSCs) within peripheral blood mononuclear cells (PBMCs) or their change at C2D1 are associated with 1yr-OS. In GBM patients, T_{reg} and MDSC populations have been reported to facilitate tumor immune evasion. T_{regs} have been found an increased fraction of the circulating CD4 compartment in GBM patients and correlates with proliferative defects among $CD4^+$ T cells.[94] MDSCs are a heterogeneous group of immature myeloid-derived cells that are capable of suppressing the immune system and are increased in the blood and tumor tissue of patients with various tumors. Recently, MDSCs were found to be increased in the blood of GBM patients compare to healthy controls, while a subset of MDSCs [polymorphonuclear MDSCs (PMN-MDSCs)] were highly prevalent in GBM tumor tissue.[95]

All subjects will have research blood drawn at baseline and at C2D1 for flow cytometry studies. 30 mL of (EDTA purple top tube) whole blood will be drawn and immediately processed at the study sites using Ficoll technique for isolation of PBMCs. Processed study samples will be frozen and stored until shipment to NYUSoM for analysis. In melanoma patients treated with nivolumab, T_{regs} decreased in responders and stable patients and significantly increased in non-responders at 12 weeks.[93] At the Immune Monitoring Core at NYUSoM, PBMCs will be thawed and analyzed by flow cytometry. Functional and phenotypic markers of T cells will be evaluated by flow cytometry using antibodies from BD Biosciences, except where indicated. PBMCs will then be stained with Live/Dead violet dye (Invitrogen) to gate on live cells. Then, cells will be assessed for expression of CD45, CD3, CD4, CD8, PD-1 (MIH4 from eBioscience), and CTLA-4.

T_{regs} will be defined as cells with $CD4^+CD25^+CD127^{low}FoxP3^+$ (eBioscience). MDSCs will be defined as MHC class II negative, $CD33^+$, $CD15^+$ cells as previously described.[95] Cells will be assessed for expression of CD33, HLA-DR, -DP, -DQ and CD15 (Becton Dickinson). Data will be acquired on an LSR II flow cytometer (BD Biosciences) and analyzed with Flowjo software (TreeStar).

5. Peripheral Blood Deep Sequencing

Objectives: Peripheral blood will be collected for deep sequencing for the purposes of:

- **A)** Sequencing normal (germline) DNA to identify tumor somatic mutations
- **B)** Assessing for alterations in TCR clonotype frequencies in pre- and post-treatment blood
- **C)** Exploring the potential of identifying circulating tumor DNA in patients with glioblastoma

B) TCR clonotype frequencies (All subjects): We will determine whether study treatment results in peripheral expansion of TCR clonotypes found in TIL clones identified by tumor tissue whole-exome sequencing and alters the TCR repertoire of the most expanded TCR clonotypes. Previous studies indicate radiotherapy and immune checkpoint inhibition activate non-redundant immune mechanisms and the major contribution of radiotherapy may be increasing TCR diversity and shaping the TCR repertoire of the expanded peripheral T cell clones.[37] DNA will be extracted from peripheral blood cells and analyzed with whole-exome deep sequencing to analyze frequencies/counts of the top 100 most frequent TCR clonotypes identified in baseline tumor tissue.

C) Circulating tumor DNA in patients with GBM*:* We will sequence cell-free fractions of blood to high depths to attempt to detect circulating mutant *TERT* allele and also attempt to compare the changes in the allele fraction between baseline and post-treatment blood.

All subjects will have research blood drawn at baseline and at C2D1. 10 mL of whole blood in EDTA tubes (purple top tubes) will be drawn at each timepoint and processed. From the whole blood sample, a 1mL aliquot will be immediately taken and frozen separately in a cryovial. The remaining sample will be spun to separate the plasma, buffy coat, and cells. The buffy coat and plasma will each be separated and placed in cyrovials for freezing. Samples will be batch-shipped to the **NYUSoM Center for Biospecimen Research and Development (CBRD)**. DNA will be extracted and analyzed with whole-exome deep sequencing to analyze frequencies/counts of the top 100 most frequent TCR clonotypes identified in baseline tumor tissue.