Abbreviated Title: Cytokine Microdialysis

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Title: Cytokine microdialysis for real time immune monitoring in glioblastoma patients undergoing checkpoint blockade

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Investigational Agents:

Drug Name:	Nivolumab (BMS-936558)
IND Number:	136039
Sponsor:	NINDS
Manufacturer:	Bristol-Myers Squibb (BMS)

Drug Name:	Anti-LAG-3 (BMS-986016)
IND Number:	136039
Sponsor:	NINDS
Manufacturer:	Bristol-Myers Squibb (BMS)

Multi-Institutional Protocol Coordinating Center: National Institute of Neurological Disorders and Stroke

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Précis:

Objective

This protocol is being performed to 1) characterize the clinical and 2) immunological response of patients with recurrent glioblastoma to treatment with Nivolumab, together with an anti-Lag-3 antibody, BMS-986016, and to evaluate the safety of brain tumor microdialysis in this patient population.

Study Population

10 patients (total, after replacement for any dropout), 18 years old and older with recurrence of glioblastoma after standard treatment of surgery, chemotherapy, and radiation.

Study Design

Patients will be screened by study neurosurgeons or neuro-oncologists to verify their confirmed or likely diagnosis of a recurrent glioblastoma. Patients will be offered standard of care therapy, including repeat surgery and/or recommendations for chemotherapeutic agents and other trials. If the patients are deemed to be surgical candidates for their potential recurrence, they will be enrolled in the trial. Enrolled patients will then undergo a stereotactic brain biopsy. If a frozen section confirms a diagnosis of recurrent glioblastoma, two microdialysis catheters will be placed in the brain after the biopsy, and a lumbar drain will also be placed. These microdialysis catheters will sample interstitial fluid in and around the brain tumor every 6 hours. We will collect blood and cerebral spinal fluid samples daily for comparison. After two days (Day 3), the patients will be given one dose of Nivolumab, 240mg IV. We will continue to collect samples every six hours from the microdialysis catheters and daily from blood and cerebral spinal fluid for 5 additional days, after which patients will undergo surgical resection of their tumors and removal of the microdialysis catheters and lumbar drain. Nivolumab, at a dose of 240mg IV over 30 minutes every 2 weeks, will be administered after surgery (starting on Day 17 (+/- 2 days), two weeks after the first dose on Day 3) followed by BMS 986016, an anti-Lag-3 antibody at a dose of 80mg IV over 60 minutes, until the study neuroradiologist notes tumor progression on MRI or the patient experiences treatment toxicity. While on therapy with Nivolumab and BMS-986016, patients will be seen and examined every 2 weeks +/- two days for signs of toxicity. Patients will be followed for at least three months after the surgical procedure.

Outcome Measures

The primary outcome measures are the proportion of patients who have a measurable increase of interferon gamma levels in the brain tumor tissue after their first dose of Nivolumab as compared to the pre-treatment baseline, the safety of using brain tumor microdialysis to monitor response to immune modulators in patients with recurrent

glioblastoma and the safety of the combination of Nivolumab and BMS-986016.
Exploratory outcome measures include:
1) To determine the change in interferon gamma production within the tumor microenvironment and in the rest of the body from before and after therapy with the immune checkpoint inhibitor, nivolumab; 2) To evaluate the pathological response of the immune microenvironment of brain tumor tissue to the first dose of Nivolumab; 3) To evaluate the clinical response (progression free survival, overall survival) of recurrent glioblastoma patients to this treatment combination; 4) To describe the difference in survival between responders and non-responders on this treatment combination; 5) To examine the differences in the immune cells and secreted factors of the tumor environment as compared to the immune cells and secreted factors of the cerebral spinal fluid, blood and, potentially, bone marrow in response to this treatment.

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List of Abbreviations

AE	Adverse Events
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
AST	Aspartate Transaminase
AT	Aminotransferase
BMS	Bristol-Myers Squibb
CD3,4, 8	Cluster of Differentiation 3, 4, 8, etc
CL	Confidence Limits
CNS	Central nervous system
CRFs	Case Report Forms
CSF	Cerebral Spinal Fluid
CT	Computer-assisted Tomography
CTLA-4	Cytotoxic T-Lymphocyte Associated protein 4
DILI	Drug Induced Liver Injury
DoH	Declaration of Helinski
DPA	Durable Power of Attorney
DSMB	Data and Safety Monitoring Board
DTI	Diffusion Tensor Imaging
FDA	Food and Drug Administration
FOXP3	Forkhead box P3
FWA	Federalwide Assurance
GDS	Genomic Data Sharing
ICU	Intensive Care Unit
IFNg	Interferon Gamma
IL2	Interleukin-2
IMM	Independent Medical Monitor
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
LAG3	lymphocyte activation gene 3
MRI	magnetic resonance imaging
NCI	National Cancer Institute
NINDS	National Institute of Neurological Disorders and Stroke
NOB	Neuro-Oncology Branch
NPCU	Neuroscience Patient Care Unit
PD-1	Programmed Death 1
PD-L1	Programmed Death Ligand 1
PI	Principle Investigator
PRPL	Patient Recruitment Public Liaison
QA	Quality Assurance
RANO	Response Assessment in Neuro-Oncology
SAE	Serious Adverse Event
SMC	Safety Monitoring Committee
SNB	Surgical Neurology Branch
TGFB-2	Transforming Growth Factor Beta-2

TNF	Tumor Necrosis Factor
ULN	Upper Limit of Normal

1.0 Introduction

1.1 Purpose of the Project and Scientific Justification

This protocol is a pilot study being performed to 1) determine the response of the brain's immune system to treatment with Nivolumab in patients that have a recurrence of the glioblastoma 2) Determine the safety of the use of Nivolumab together with an anti-Lag-3 antibody, BMS-986016 , in recurrent glioblastoma patients 3) determine the difference in response between the brain and the peripheral immune system to this drug 4) evaluate the response of recurrent glioblastoma patients to this combination treatment 5) determine whether immune factors will predict whether a patient will respond to this immune treatment 6) examine the differences in the immune cells and secreted factors of the tumor environment as compared to the immune cells and secreted factors of the cerebral spinal fluid, blood and, potentially, bone marrow 7) evaluate the safety of using brain tumor microdialysis to monitor response to immune modulators in patients with recurrent glioblastoma.

1.2 Standard of care for recurrent glioblastoma patients

Glioblastoma is the most common primary malignancy of the brain, and the prognosis with conventional therapy is poor, with a median survival of only 14.6 months after standard chemotherapy and radiation[1]. After the recent failure of bevacizumab to show consistent efficacy in trials while treating recurrent glioblastoma, there is no established standard of care for these patients[2]. The Joint Tumor Section of the American Association of Neurological Surgeons and Congress of Neurological Surgeons has attempted to compile guidelines for the treatment of recurrent or progressive glioblastoma. Per their guidelines, there is Level II evidence to support cytoreductive surgery in such patients as planned in this trial[3]. There is only Level III evidence to support radiotherapy in selected patients for local control when the target tumor is amenable for additional radiotherapy[4]. As such, at the time of recurrence, clinical trials often represent the best treatment option for a recurrent glioblastoma patient. For chemotherapeutic agents, there is level II evidence for the use of temozolomide when the patient did not receive temozolomide at initial diagnosis[5]. This is increasingly rare, as temozolomide has been established as standard of care for newly diagnosed glioblastoma patients. The committee has also supported the use of use of BCNU-impregnated wafers as a surgical adjunct in selected patients who undergo surgical resection with Level II evidence. These wafers, implanted into the resection cavity of glioblastoma patients, were approved by the FDA after a study showed that median survival was 31 weeks after implantation, but the placebo arm received no therapy other than placebo wafers[6]. Furthermore, the drug was approved prior to the establishment of temozolomide as standard of care, and further studies have shown significant side effects from the use of these wafers, without significant increases in survival, and as such, they are not currently widely used[7]. Bevacizumab has also been approved by the FDA for treatment of recurrent glioblastoma patients. However, recently, a large European trial showed that this treatment does not appear to be effective in increasing overall survival in recurrent glioma patients, and as such, there no longer is consensus that this is the best treatment for patients at recurrence[2]. Given the lack of high quality evidence for any treatment for

recurrent glioblastoma patients, there is not any strong recommendation for one established therapy for recurrent glioblastoma patients. The committee presented a recommendation that clinical trials be considered for recurrent glioblastoma patients, after individual patient characteristics have been taken into account.

1.3 Immune therapy – Background

The immune system constantly samples and interrogates the cells of the body to detect abnormal proteins associated with neoplastic transformation, which mark the neoplastic cells for destruction. When it is working well, immune surveillance keeps neoplastic processes at bay, whereas impaired immune function can enhance the risk of tumor development. For this reason, immune therapy has been attractive for treating cancerous processes, going back to Coley's toxin[8], a mixture of killed bacteria employed in 1893 as a vaccine to exploit a previously observed relationship between infection and cancer regression. Despite the initial enthusiasm and theoretical promise, immune therapy for cancer has largely been pushed to the wayside by chemotherapy and radiation until recently.

Many years of study into the microenvironment of malignant tumors has shown that many of these lesions produce immunosuppressive factors that keep the immune system in check and unable to detect the tumor, allowing these lesions to continue to proliferate unchecked[9]. This occurs even though many of these lesions evoke an inflammatory influx of immune cells[10]. To enhance immune responses to malignant tumors, investigators have recently developed monoclonal antibodies that target and inactivate immunosuppressive pathways.

Programmed Death 1 (PD-1) is a T cell surface immunosuppressive receptor; it is activated by Programmed Death Ligand 1 and 2 (PD-L1 and PD-L2)[11]. PD-L1 is upregulated in the tumor microenvironment of many tumors, and engagement of this ligand with its receptor, PD-1, reduces the cellular immune response within the tumor microenvironment[11]. Previously, immune therapy employing autologous killer T-cells stimulated with interleukin 2 (IL2) resulted in tumor regression in some patients undergoing this very taxing therapy of metastatic melanoma[12]. More recently, targeting of the immunosuppressive checkpoint, PD-1, has provided initial evidence that immune therapy could be applied more broadly to other cancers. Nivolumab, a monoclonal antibody targeting PD-1 was found to be efficacious and approved[13] for advanced melanoma, as was Pembrolizumab, another PD-1 inhibitor[14]. Lymphocyte Activation Gene 3 (LAG-3) is another checkpoint present on activated T cells (Figure 1) [15]. LAG-3 and PD-1 are co-expressed on the surface of tumor infiltrating T-cells in ovarian cancer patients, and denote poorly functional CD8+ T effector cells[16]. The efficacy of single agent checkpoint inhibition may be limited by compensatory upregulation of other checkpoints, and this may be overcome by using combinations of checkpoint inhibitors[17].

1.4 Checkpoint inhibition in glioblastoma patients

One immunosuppressive factor, Transforming Growth Factor Beta-2 (TGFB-2) was first identified in lysates of glioblastoma tissues, and since then, many immunosuppressive factors have been identified within the glioblastoma microenvironment[9, 18, 19]. While there was once a concept of immune privilege in the brain, meaning that the immune system lacked effective immune surveillance due to the blood brain barrier and absence of clearly identified lymphatic channels, subsequent studies have shown this to be false[20, 21]. The brain has numerous cells engaged in constant immune surveillance, and there is widespread belief that these checkpoint inhibitors, Nivolumab and Ipilimumab, could target immunosuppression in the glioblastoma microenvironment, just as they have done in other systemic tumors. Preclinical models have supported this argument, with murine anti-CTLA-4 antibodies showing a significant treatment effect and numerous long-term survivors in an aggressive immune-competent murine glioma model system[22]. Anti PD-1 and PD-L1 antibodies were shown to be similarly effective[23, 24].

The successes of these therapeutics are not guaranteed in the human brain for numerous reasons. The murine immune system and the human immune system are very different, with different cytokine profiles and dynamics for cell infiltration and inflammation. There has been a suggestion in some cancers that PD-L1 expression on the native tumor is correlated with response to checkpoint inhibitor therapy[25], but unlike common glioma cell lines used in preclinical models, the expression of PD-L1 in the glioma microenvironment is heterogeneous and may not be as robust as once reported[26]. Beyond this, even in the successful trials of these inhibitors, these treatments seem to only be effective in a small subset of patients, and these treatments can cause widespread systemic inflammation, causing several side effects, many of them severe[27]. Systemic immunophenotyping in checkpoint inhibitor trials in other cancers has thus far yielded data of limited value in the glioblastoma population[28]. No clear predictors of response to these checkpoint inhibitors in systemic cancers have been identified. Furthermore, while we know that immune cells can infiltrate the glioblastoma microenvironment, we do not know whether systemic immune stimulation from checkpoint inhibitors will translate to a response in the immune cells that traffic to glioblastoma microenvironment. To understand the role systemic checkpoint inhibitors could play in glioblastoma therapy, research needs to be conducted to quickly and accurately determine if systemically-administered checkpoint inhibitors reach the brain tumor, immediately impact the tumor microenvironment, and evoke a therapeutic anti-tumor immune effect. This research could provide data that would predict if checkpoint inhibitor immunotherapy could be developed as a standard therapy for glioblastoma or whether we need to focus our resources elsewhere. Such due diligence of early evaluation of the therapeutic potential of a new glioblastoma therapy is particularly important in light of the recent failed trials for rindopepimut, a vaccine targeted at the epidermal growth factor variant III, in newly diagnosed patients (data not yet published), which showed great initial promise in a Phase II study[29]. A recent presentation at the American Association of Neurological Surgeons (AANS) 2016 Annual Meeting demonstrated that treatment of patients with recurrent glioblastoma with ipilimumab, or the combination of ipilimumab and nivolumab, resulted in nearly equivalent overall survival of two cohorts of 10 patients. Significantly more side effects were seen in the ipilimumab and nivolumab arm compared to the nivolumab-alone arm. A more recent presentation of the results of the Checkmate 143 trial at the 2017

World Federation of Neuro-Oncology Societies meeting demonstrated that Nivolumab did not show efficacy as a single agent in recurrent glioblastoma patients as compared to bevacizumab. While the treatment as a single agent may have been effective in certain patient subsets, preclinical data suggests that a combination of anti-PD-1 and BMS-986016 may be more efficacious in cancer models (unpublished BMS data, Table 1, Appendix C), and this approach is currently undergoing Phase I testing in glioblastoma (NCT02658981). As such, we believe that this drug combination has the potential to confer additional benefit to our patients, improving the risk/benefit ratio of the study. Both drugs are being provided for this study by the manufacturer, Bristol-Myers Squibb, at no cost.

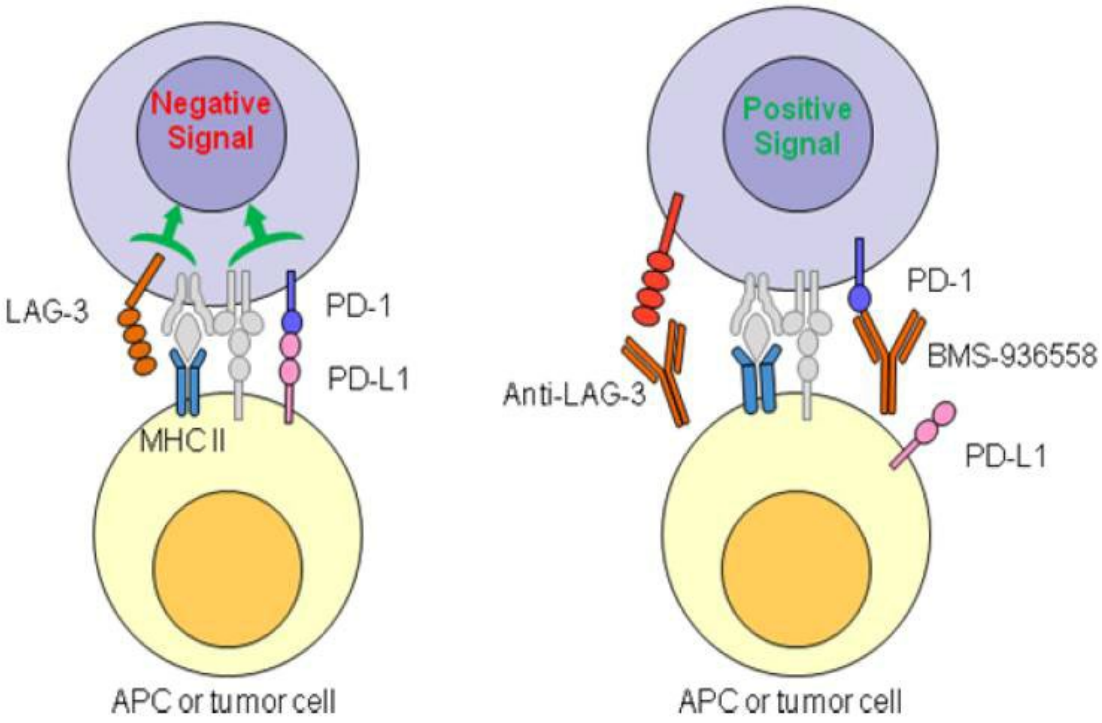

Figure 1. Model for Augmented T-cell Activity Mediated by Inhibition of LAG-3 and PD-1 (BMS-986016 Investigator Brochure, Appendix C)

Study No.	Treatment Day	Treatment Group	Tumor-free Mice[a]
BDX-1408-206	7, 10, 12	Control (IgG1) 10 mg/kg	0/10
		Anti-PD-1 (4H2) 10 mg/kg	4/10
		Anti-LAG-3 (C9B7W) 10 mg/kg	0/10
		Anti-PD-1 (4H2) 10 mg/kg + anti-LAG-3 (C9B7W) 10 mg/kg	7/10
BDX-1408-207	8, 11, 14	Control (IgG1) 20 mg/kg	0/10
		Anti-PD-1 (4H2) 10 mg/kg + IgG1 10 mg/kg	4/10
		Anti-LAG-3 (C9B7W) 10 mg/kg + IgG1 10 mg/kg	0/10
		Anti-PD-1 (4H2) 10 mg/kg + anti-LAG-3 (C9B7W) 10 mg/kg	8/10
		Anti- CTLA-4 (9D9) 10 mg/kg + IgG1 10 mg/kg	0/10
		Anti-CTLA-4 (9D9) 10 mg/kg + anti-LAG-3 (C9B7W) 10 mg/kg	3/10

Source: DCN 930054255 (Study BDX-1408-206)[41] and DCN 930071268 (Study BDX-1408-207)[42]

[a] Number of tumor-free mice at the end of the study/total number of mice in group

Table 1: Number of Tumor-free Mice at End of Study by Treatment Group: MC38 Tumor Model Studies (BMS-986016 Investigator Brochure, Appendix C)

1.5 Pharmacology of Nivolumab

1.5.1 Introduction

Nivolumab (also referred to as Opdivo®, BMS-936558, MDX1106, or ONO-4538) is a human monoclonal antibody (HuMAb; 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. Nivolumab is approved for the treatment of several types of cancer in multiple regions including the United States (US, Dec-2014), the European Union (EU, Jun-2015), and Japan (Jul-2014). Nivolumab is also being investigated in various other types of cancer as monotherapy or in combination with other therapies, and as single-dose monotherapy for the treatment of sepsis.

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-release.

1.5.2 Non-clinical Studies

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)] 117,000g•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.

1.5.3 Effects in Humans

The pharmacokinetics (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), classical Hodgkin Lymphoma (cHL), urothelial carcinoma (UC), squamous cell carcinoma of the head and neck (SCCHN), in addition to other tumor types. The Investigator Brochure (IB) references the most recent US Prescribing Information (USPI) and EU Summary of Product Characteristics (SmPC) as the basis for the current state of knowledge on nivolumab for use treating cancer in humans.

Nivolumab monotherapy is approved in multiple regions, including the US and EU, for unresectable or metastatic melanoma, previously treated metastatic NSCLC, previously treated advanced RCC, previously treated relapsed or refractory cHL, and previously treated advanced or metastatic UC; it is also approved for the treatment of previously treated recurrent or metastatic SCCHN 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 for the treatment of several types of cancer. Single- dose nivolumab monotherapy is also being investigated for the treatment of sepsis. Additional clinical activity and safety information presented in the IB

focus primarily on data from clinical studies that are relevant to ongoing clinical investigations not in the approved USPI and SmPC (small cell lung cancer [SCLC]), gastric cancer, hepatocellular carcinoma [HCC], colorectal cancer [CRC], glioblastoma [nivolumab monotherapy], and Merkel cell carcinoma [MCC]; SCLC, gastric and esophageal cancers, NSCLC, RCC, and CRC [nivolumab combination therapy]; and Ono

1.5.4 Nivolumab Drug Description, Prep and Storage Conditions

Nivolumab, also referred to as BMS-936558-01 or BMS-936558, is a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains. The physical and chemical properties of nivolumab are provided in Table 3.1-1 of the Nivolumab Investigator's brochure (included below).

Table 3.1-1: Physical and Chemical Properties

BMS Number	BMS-936558-01
Other Names	Opdivo®, nivolumab, BMS-936558, MDX1106, ONO-4538, anti-PD-1
Molecular Weight	146,221 daltons (143,619.17 daltons, protein portion)
Appearance	Clear to opalescent, colorless to pale yellow liquid, light (few) particulates may be present
Solution pH	5.5 to 6.5

Drug Preparation

Nivolumab Injection, 100 mg/10 mL (10 mg/mL) and 40 mg/4 mL (10 mg/mL)

Vials of nivolumab injection must be stored at 2°C to 8°C (36°F to 46°F) and protected from light and freezing.

Nivolumab injection 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 and infusion times. It is not to be administered as an IV push or bolus injection. When the dose is based on patient weight (ie, mg/kg), 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. When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 120 mL.

During drug product preparation and handling, vigorous mixing or shaking is to be avoided. Instructions for dilution and infusion of nivolumab injection may be provided in the clinical protocol, pharmacy binder, pharmacy manual, or pharmacy reference sheet. 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.

Storage conditions

Nivolumab Injection, 100 mg/10 mL (10 mg/mL) and 40 mg/4 mL (10 mg/mL)

Vials of nivolumab injection must be stored at 2°C to 8°C (36°F to 46°F) and protected from light and freezing.

Undiluted Nivolumab Injection and Diluted Nivolumab Injection in the IV Container
The administration of nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored under refrigeration conditions (2°C to 8°C, 36°F to 46°F) for up to 24 hours, and a maximum of 8 hours of the total 24 hours can be at room temperature (20°C to 25°C, 68°F to 77°F) and room light. The maximum of 8 hours under room temperature and room light conditions includes the product administration period.

First Nivolumab dose preparation and administration

Nivolumab will be administered first. As with all injectable drugs, care should be taken when handling and preparing Nivolumab. Whenever possible, Nivolumab infusions should be prepared in a laminar flow hood, glovebox, or safety cabinet using standard procedures for the safe handling of intravenous agents applying aseptic techniques. Gloves are required. If nivolumab solution comes in contact with the skin or mucosa, immediately and thoroughly wash with soap and water.

Visually inspect drug product solution for particulate matter and discoloration prior to administration. Nivolumab is a clear to opalescent, colorless to pale yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.

Preparation

To prepare 240mg dose:

1. Withdraw the required volume of Nivolumab and transfer into a 50 mL 0.9% Sodium Chloride, USP or 5 % Dextrose for Injection, USP bag. Infusion final volume for flat dosing should not exceed 120 mL in total volume.

2. Mix diluted solution by gentle inversion, do not shake.

3. Discard partially used vials or empty vials of Nivolumab.

Storage of Infusion

The product does not contain a preservative. After preparation, store the Nivolumab infusion either:

- at room temperature for no more **than 8 hours** from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion

or

- under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of infusion preparation.

  Do not freeze.

Administration

Administer the infusion over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line filter (pore size of 0.2 micrometer to 1.2 micrometer). Do not coadminister other drugs through the same intravenous line. **Flush the intravenous line at end of infusion with appropriate amount of diluent (e.g. 15-20 ml) to ensure that the total dose is administered. Total infusion and flush time should equal to 30 minutes.**

Nivolumab should not be infused concomitantly in the same intravenous line with other agents. No physical or biochemical compatibility studies have been conducted to evaluate the co-administration of Nivolumab with other agents.

First Nivolumab dose infusion monitoring

Monitoring for an infusion reaction and cytokine release on day 3 of the protocol will consist of clinical observation with q15 minute vital signs, including temperature, during and for the first hour after infusion. Specifically, we will be checking the patients for fever, chills, shakes, itching, rash, hypertension or hypotension or difficulty breathing during the administration or immediately afterwards (within the following 2 hours). The patients will be treated according to I-O AE Algorithms from Appendices B and C; Investigator Brochures for Nivolumab (BMS-936558) and Relatlimab (BMS-986016). Generally, Grade 1 infusion reactions will be observed. Grade 2 or 3 infusion reactions will prompt interruption of the patient's infusion until the observed symptom returns to the level of a Grade 1 reaction. Patients will be given 25mg of Benadryl IV and 650 mg of Tylenol PO for any grade 2 or 3 reaction. The Benadryl can be repeated once for symptom resolution until the reaction returns to a level of Grade 1. If a patient has a Grade 2 or 3 reaction, subsequent infusions will be preceded by 25mg of Benadryl IV and 650mg of Tylenol PO 30 minutes prior to dosing. Grade 4 reactions will cause discontinuation of the therapy. Please see section 1.6.5 for monitoring of combined infusions, below.

1.5.5 Clinical Pharmacokinetics

The Pharmacokinetics (PK) of nivolumab was studied in subjects with cancer over a dose range of 0.1 to 20 mg/kg administered as a single dose or as multiple doses of nivolumab every 2 or 3 weeks. Nivolumab clearance (CL) decreases over time, with a mean maximal reduction (% coefficient of variation [CV%]) from baseline values of approximately 24.5% (47.6%) resulting in a geometric mean steady state clearance (CLss) (CV%) of 8.2 mL/h (53.9%); the decrease in CLss is not considered clinically relevant. The geometric mean volume of distribution at steady state (Vss) was 6.8 L (27.3%), and geometric mean elimination half-life (t1/2) was 25 days (77.5%). Steady-state concentrations of nivolumab were reached by 12 weeks when administered at 3 mg/kg Q2W, and systemic accumulation was approximately 3.7-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 status, solid tumor type, baseline tumor size, and hepatic impairment.

Although ECOG status, baseline glomerular filtration rate (GFR), albumin, and body weight had an effect on nivolumab CL, the effect was not clinically meaningful. PPK analysis suggest that nivolumab CL in subjects with cHL was approximately 32% lower relative to subjects with NSCLC; however, the lower CL in cHL subjects was not considered to be clinically relevant as nivolumab exposure was not a significant predictor for safety risks for these patients. When nivolumab is administered in combination with ipilimumab, the CL of nivolumab was increased by 29%, whereas there was no effect on the clearance of ipilimumab.

PPK and exposure response analyses have been performed to support use of nivolumab 240 mg Q2W, 360 mg Q3W, and 480 mg Q4W dosing regimens in subjects with cancer in addition to the 3 mg/kg Q2W regimen. A flat dose of nivolumab 240 mg Q2W was selected since it is identical to a dose of 3 mg/kg for subjects weighing 80 kg, the observed median body weight in nivolumab- treated cancer patients, while the nivolumab 360 mg Q3W and 480 mg Q4W regimens allow flexibility of dosing with less frequent visits and in combination with other agents using alternative dosing schedules to Q2W. Using a PPK model, the overall distributions of nivolumab exposures (Cavgss, Cminss, Cmaxss, and Cmin1) are comparable after treatment with either nivolumab 3 mg/kg or 240 mg Q2W. Following nivolumab 360 mg Q3W and 480 mg Q4W, Cavgss are expected to be similar to those following nivolumab 3 mg/kg or 240 mg Q2W, while Cminss are predicted to be 6% and ~16% lower, respectively, and are not considered to be clinically relevant. Following nivolumab 360 mg Q3W and 480 mg Q4W, Cmaxss are predicted to be approximately ~23% and ~43% greater, respectively, relative to that following nivolumab 3 mg/kg Q2W dosing. However, the range of nivolumab exposures (median and 90% prediction intervals) following administration of 240 mg flat Q2W, 360 mg Q3W, and 480 mg Q4W regimens across the 35 to 160 kg weight range are predicted to be maintained well below the corresponding exposures observed with the well tolerated 10 mg/kg nivolumab Q2W dosing regimen

The PK of single-dose nivolumab monotherapy in subjects with sepsis is under evaluation; however, no data are currently available.

Administration of nivolumab using a 30-minute infusion time has been evaluated in subjects with cancer. Previous clinical studies of nivolumab monotherapy for the treatment of cancer have used a 60-minute infusion duration wherein nivolumab has been safely administered up to 10 mg/kg over long treatment periods. Infusion reactions including high-grade hypersensitivity reactions have been uncommon across the nivolumab clinical program. In CA209010, a dose association was observed for infusion site reactions and hypersensitivity reactions (1.7% at 0.3 mg/kg, 3.7% at 2 mg/kg and 18.5% at 10 mg/kg). All the events were Grade 1-2 and were manageable. An infusion duration of 30 minutes for 3 mg/kg nivolumab (30% of the dose provided at 10 mg/kg) is not expected to present any safety concerns compared to the prior experience at 10 mg/kg

nivolumab dose infused over a 60-minute duration. The safety of nivolumab 3 mg/kg administered as a 30-min infusion was assessed in CA209153 in patients (n=322) with previously treated advanced NSCLC. Overall, there were no clinically meaningful differences in the frequency of hypersensitivity/infusion-related reactions (of any cause or treatment-related) in cancer patients administered nivolumab over a 30-min infusion compared with that reported for patients with the 60-min infusion. Thus, it was shown that nivolumab can be safely infused over 30 min in subjects with cancer.

1.5.6 Clinical Efficacy

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.

1.5.7 Clinical Safety

The overall safety experience with nivolumab is based on experience in approximately 23,507 subjects as either monotherapy or in combination with other therapeutics. In general, for monotherapy, the safety profile is similar across tumor types. The only exception is pulmonary inflammation AEs, which may be numerically greater in subjects with NSCLC, possibly because in some cases, it can be difficult to distinguish between nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes. The most frequently reported treatment-related AE is fatigue, which is almost always of low grade.

Most related AEs are thought to be due to the effects of inflammatory cells on specific tissues. A variety of preferred terms (PTs) have been used to describe similar kinds of organ-related AEs, with the result being that AE frequency tables organized by PTs can lead to underestimation of the frequency of similar kinds of organ-related AEs. To address this issue, select AE categories were created. Select AE categories group together the most common and impactful PTs by organ category. These categories include the following: pulmonary, GI, hepatic, skin, endocrine, hypersensitivity/infusion reaction, and renal AEs. The frequency of select AE categories is provided in the sub-sections for individual indications in the investigator’s brochure. It is also useful to consider the management of nivolumab-related AEs by organ category as the diagnostic work-up often requires excluding other potential diagnoses and, when appropriate, instituting specific management principles as outlined in the investigator’s brochure.

1.6 Pharmacology of BMS-986016

1.6.1 Introduction

BMS-986016 is a human lymphocyte activation gene 3 (LAG-3)-specific antibody that is isolated after immunization of transgenic mice expressing human immunoglobulin (Ig) genes. BMS-986016 has been given the generic name of relatlimab. It is expressed as an IgG4 isotype antibody and includes a stabilizing hinge mutation (S228P). BMS-986016 binds to LAG-3 with high affinity and inhibits the negative regulatory function of LAG-3 in vitro. Binding of BMS-986016 to LAG-3 prevents binding of this receptor to cells bearing its ligand, major histocompatibility complex (MHC) Class II, the peptide antigen presentation molecule recognized by CD4+ T cells. By blocking the normal downregulatory pathway, BMS-986016 enhances the anti-tumor immune response and, thus, has the potential to inhibit the growth of multiple malignancies when administered as a single agent or in combination with other therapeutic immuno-oncology (IO) monoclonal antibodies.

1.6.2 Nonclinical Characterization

BMS-986016 binds to human (half maximal effective concentration [EC50] = 0.11 nM) and cynomolgus monkey (EC50 = 29.11 nM) LAG-3 but does not bind to mouse LAG-3. Using surrogate antibodies recognizing mouse LAG-3 (C9B7W and 19C7), anti-tumor activity has been demonstrated in 3 murine syngeneic in vivo tumor models (Sa1N fibrosarcoma, MC38 colon adenocarcinoma, and A20 B-cell lymphoma). Both tumor inhibition and the number of tumor-free mice were increased by BMS-986016 monotherapy, while the combination of BMS-986016 with a blocking anti-programmed cell death protein 1 (PD-1) antibody provided enhanced anti-tumor activity higher than the activity of either agent alone and higher than the activity of anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibody in some subjects.

In a repeat-dose study in monkeys, following weekly intravenous (IV) doses of BMS-986016 monotherapy (30 and 100 mg/kg) or dosed in combination with nivolumab (100 mg/kg of BMS-986016 and 50 mg/kg of nivolumab [an anti-PD-1 antibody]) for 4 weeks, the total serum clearance (CLT) of BMS-986016 was 0.12 mL/h/kg, and the elimination half-life (T-HALF) was 414 hours (estimated by population pharmacokinetic [PK] analysis of pooled data from all 3 groups). There was no apparent PK drug-drug interaction between BMS-986016 and nivolumab.
Based on the BMS-986016 NOAEL determined from the GLP-compliant toxicity study (100 mg/kg/week IV), the maximum IV dose provides a safety multiple of approximately 10 times for both the maximum observed concentration (Cmax) and AUC(0-336h) after the first 800-mg dose. Overall, the nonclinical toxicology assessment of BMS-986016 has demonstrated an acceptable profile, supporting its continued clinical use in humans.

1.6.3 Effects in Humans

As of the clinical cutoff date of 29-Jun-2016, 89 subjects have been treated with BMS-986016 in 2 ongoing studies assessing PK, clinical activity, and safety. The current Phase 1 clinical program is evaluating advanced solid tumors (special focus in non-small cell lung cancer [NSCLC], renal cell carcinoma, and malignant melanoma) in Study CA224-020 and relapsed-refractory hematological malignancies (Hodgkin and non-Hodgkin lymphomas) in Study CA224-022. BMS-986016 is being investigated both as monotherapy and in combination with nivolumab. As of 29-Jun-2016, BMS-986016 monotherapy has been administered to 60 subjects, as follows: 8 subjects at 20 mg, 13 subjects at 80 mg, 24 subjects at 240 mg, and 15 subjects at 800 mg, all receiving a flat dose on a biweekly (q2w) regimen. Twenty-two of these subjects were from Study CA224-020, and 38 subjects were from Study CA224-022. As of 29-Jun-2016, combination therapy with BMS-986016 and nivolumab has been administered to 29 subjects, as follows: 7 subjects at 20 mg BMS-986016/80 mg nivolumab and 9 subjects at 20 mg BMS-986016/240 mg nivolumab, 9 subjects at 80 mg BMS-986016/240 mg nivolumab, and 4 subjects at 240 mg BMS-986016/240 mg nivolumab, with all regimens at a flat dose q2w. Safety information presented in this Investigator Brochure (IB) focuses primarily on information obtained from Phase 1 studies at 4 ascending doses of BMS-986016 monotherapy (flat doses of 20, 80, 240, and 800 mg) from Studies CA224-020 and CA224-022 and 4 ascending doses of BMS-986016 and nivolumab combination therapy (flat doses of 20 mg BMS-986016/80 nivolumab, 20 mg BMS-986016/240 mg nivolumab, 80 mg BMS-986016/240 mg nivolumab, and 240 mg BMS-986016/240 mg nivolumab) from Study CA224-020.

1.6.4 BMS-986016 Drug Description, Prep and Storage Conditions

BMS-986016, also referred to as BMS-986016-01 or anti-LAG-3, was selected for dosage form development. BMS-986016 is a soluble protein consisting of 4 polypeptide chains that include 2 identical heavy chains and 2 identical light chains and is a human IgG4 monoclonal antibody directed against human LAG-3. BMS-986016 is produced from cell culture using a Chinese Hamster Ovary (CHO) cell line. The physical and chemical properties of BMS-986016 drug substance are summarized in the table below.

Table 2: Physical and Chemical Properties of BMS-986016 Drug Substance

BMS Number	BMS-986016-01
Other Names	Anti-LAG-3; BMS-986016
Molecular Weight	147,179 Daltons
Appearance	Clear to opalescent, colorless to pale yellow liquid, may contain particles
Solution pH	5.0 to 6.0

BMS-986016 Injections

BMS-986016 injections are to be administered as an IV infusion through a 0.2/0.22-µm 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. BMS-986016 injection can be diluted with 0.9% sodium chloride injection (normal saline) to protein concentrations no lower than 0.33 mg/mL. Detailed instructions for drug product dilution and administration are provided in the pharmacy manual for the clinical study.

Care must be taken to assure sterility of the prepared solution, as the product does not contain any anti-microbial preservative or bacteriostatic agent. No incompatibilities between BMS-986016 injection (without the pentetic acid) and polyolefin bags or non-di(2-ethylhexyl)phthalate (DEHP) infusion set have been observed. No incompatibilities between BMS-986016 (with the pentetic acid) and polyolefin, polyvinyl chloride (PVC) bags, or non-DEHP infusion set have been observed.

Recommended Storage Conditions

The drug products should be stored at 2°C to 8°C (36°F to 46°F) with protection from light. Donot freeze the drug product. The administration of BMS-986016 infusions and the co-administration of BMS-986016 and nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored in a refrigerator at 2°C to 8°C (36°F to 46°F) for up to 24 hours and a maximum of 4 hours of the total 24 hours can be at room temperature (20°C to 25°C; 68°F to 77°F) and exposed to room light. The maximum 4-hour period under room temperature and room light conditions includes the product administration period.

1.6.5 Handling and Dose Preparation for Nivolumab/BMS-986016 Administration

Nivolumab Handling and Dose Preparation

Nivolumab will be administered first. As with all injectable drugs, care should be taken when handling and preparing Nivolumab. Whenever possible, Nivolumab infusions should be prepared in a laminar flow hood, glovebox, or safety cabinet using standard procedures for the safe handling of intravenous agents applying aseptic techniques. Gloves are required. If nivolumab solution comes in contact with the skin or mucosa, immediately and thoroughly wash with soap and water.

Dose Preparation and Administration

Visually inspect drug product solution for particulate matter and discoloration prior to administration. Nivolumab is a clear to opalescent, colorless to pale yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.

Preparation

To prepare 240mg dose:

1. Withdraw the required volume of Nivolumab and transfer into a 50 mL 0.9% Sodium Chloride, USP or 5 % Dextrose for Injection, USP bag. Infusion final volume for flat dosing should not exceed 120 mL in total volume.

2. Mix diluted solution by gentle inversion, do not shake.

3. Discard partially used vials or empty vials of Nivolumab.

Storage of Infusion

The product does not contain a preservative. After preparation, store the Nivolumab infusion either:

- at room temperature for no more **than 8 hours** from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion

or

- under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of infusion preparation.

  Do not freeze.

Administration

Administer the infusion over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line filter (pore size of 0.2 micrometer to 1.2 micrometer). Do not coadminister other drugs through the same intravenous line. **Flush the intravenous line at end of infusion with appropriate amount of diluent (e.g. 15-20 ml) to ensure that the total dose is administered. Total infusion and flush time should equal to 30 minutes.**

Following completion of the nivolumab infusion, BMS 986016 should be administered. Preferably, the infusion of BMS 986016 should begin within 15 to 30 minutes after the end of the Nivolumab infusion.

Nivolumab should not be infused concomitantly in the same intravenous line with other agents. No physical or biochemical compatibility studies have been conducted to evaluate the co-administration of Nivolumab with other agents.

1.6.6 BMS-986016 handling and Dose Preparation following Nivolumab administration

As above, BMS-986016 infusion will be timed for 15-30 minutes after the completion of Nivolumab dosing. As with all injectable drugs, care should be taken when handling and preparing BMS-986016. Whenever possible, BMS-986016 infusions should be prepared in a laminar flow hood, glovebox, or safety cabinet using standard procedures for the safe handling of intravenous agents applying aseptic techniques. Gloves are required. If

BMS-986016 solution comes in contact with the skin or mucosa, immediately and thoroughly wash with soap and water.

Dose Preparation and Administration

Visually inspect drug product solution for particulate matter and discoloration prior to administration. BMS-986016 is a clear to slightly opalescent, colorless to pale yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.

Preparation

- Withdraw the required volume of BMS-986016 and transfer into an intravenous container. BMS-986016 80 mg dose (8 ml BMS-986016 10 mg/ml solution) may be mixed with 52 ml NS to a total volume = 60 ml which can be infused over 60 minutes at 1 ml/min followed by flush.

  Note: No incompatibilities between BMS-986016 injection and polyolefin or Non-DEHP IV components have been observed.

- Mix diluted solution by gentle inversion. Do not shake.
- Discard partially used vials or empty vials of BMS-986016

Storage of Infusion

The product does not contain a preservative. After preparation, store the BMS-986016 infusion either:

- at room temperature for no more than 4 hours from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion

or

- under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of infusion preparation. **Do not freeze.**

  Note: the time needed for room temperature storage of the infusion in the IV container and time for administration of the infusion (up to 4 hours) must be subtracted from this 24 hour period. For example, a 1-hour infusion and 1-hour room temperature handling and storage will result in no more than 22 hours allowed under refrigeration storage.

Administration

Administer the infusion over 60 minutes through an intravenous line containing a sterile, non-pyrogenic, polyethersulfone low protein binding in-line filter (pore size of 0.2/0.22

micrometer). Do not co-administer other drugs through the same intravenous line. **Flush the intravenous line at end of infusion with 0.9% Sodium Chloride Injection, USP (15-20 ml) to ensure that the total dose is administered. Total infusion and flush time should equal to 60 minutes.**

NOTE: When dosing sequentially with Nivolumab, the BMS-986016 infusion should begin within 15 to 30 minutes after the end of the Nivolumab infusion.

1.6.7 Infusion monitoring for combination of Nivolumab and BMS-986016

Monitoring for an infusion reaction and cytokine release on Day 17 (+/- 2 days) of the protocol will consist of clinical observation in the day hospital ward or 7SW of the NIH Clinical Center. The total infusion monitoring time will be expected to last 4-5 hours for most patients. First, the patient will receive Nivolumab as an IV infusion over 30 minutes. Patients with a prior Grade 2 or 3 infusion reaction will have pretreatment with 25mg of Benadryl IV together with 650mg of Tylenol PO. During both infusions, patients will have q15 minute vital signs, including temperature, pulse, blood pressure and pulse oximetry. BMS-986016 infusion will begin 15-30 minutes after completion of the Nivolumab infusion. BMS-986016 will then be given as an IV infusion over 60 minutes with ongoing monitoring. The patients will also be monitored for two hours after completion of the BMS-986016 in the day hospital or 7SW. Specifically, we will be checking the patients for fever, chills, shakes, itching, rash, hypertension or hypotension or difficulty breathing during the administration or immediately afterwards (within the following 2 hours). The patients will be treated according to I-O AE Algorithms from Appendices B and C; Investigator Brochures for Nivolumab (BMS-936558) and Relatlimab (BMS-986016). Generally, Grade 1 infusion reactions will be observed. Grade 2 or 3 infusion reactions will prompt interruption of the patient's infusion until the observed symptom returns to the level of a Grade 1 reaction. Patients will be given 25mg of Benadryl IV and 650 mg of Tylenol PO for any grade 2 or 3 reaction. The Benadryl can be repeated once for symptom resolution until the reaction returns to a level of Grade 1. If a patient has a Grade 2 or 3 reaction, subsequent infusions will be preceded by 25mg of Benadryl IV and 650mg of Tylenol PO 30 minutes prior to dosing. Grade 4 reactions will cause discontinuation of the therapy. Patients will be instructed to inform the clinical team of any symptoms including, but not limited to, fever, chills, itching, shortness of breath, stomach discomfort, diarrhea, new neurological symptoms, seizures, changes in urinary function, chest pain, palpitations, rashes or any other significant concern in between drug doses.

1.6.8 Clinical Pharmacokinetics

An interim determination of BMS-986016 multiple dose PK was carried out using all available serum concentration data from Studies CA224020 and CA224022. Noncompartmental analysis was performed using concentration-time data after the first dose (Cycle 1) and ninth dose (Cycle 3). In general, the maximum observed concentration (Cmax) and area under the concentration versus time curve over the dosing interval (AUC[TAU]) values over the first dosing interval increased approximately proportional to the increment in the BMS-986016 dose. BMS-986016 accumulation from

the first to ninth doses of a Q2W regimen was around 2- to 3-fold for AUC(TAU) and 1.2- to
1.8-fold for Cmax. BMS-986016 effective half-life was estimated to be approximately 20 days. The PK of BMS-986016 or nivolumab were not altered when given in combination.

BMS-986016 population PK was best described by a 2 compartment model with parallel linear and non-linear CL. The linear portion represents the non-specific clearance (CL), and non-linear component represents target-mediated CL. The linear CL was 0.18 L/day, and the volume of distribution in the central compartment was 4.5 L. The maximum rate of non-linear elimination (Vmax) was 2.5 mg/day, and the concentration that achieved 50% of Vmax (Km) was 5.7 μg/mL.

Currently available data suggest that BMS-986016 monotherapy exhibits a low level of immunogenicity, with 6 out of 42 subjects having at least 1 post-baseline positive ADA samples. There are limited data available in combination cohort to make inference on immunogenicity rate.

1.6.9 Clinical Safety

As of the clinical cutoff date of 15-Jul-2020, treatment with BMS-986016 in combination with nivolumab has been administered to 1332 subjects. Treatment-related AEs were reported in 890 (66.8%) subjects, with the most commonly reported (≥ 5% of subjects) being fatigue (15.5%), pruritus (7.4%), diarrhea (8.6%), asthenia (7.4%), rash (7.1%), arthralgia (7.5%), hypothyroidism (7.2%) and increased lipase (6.5%). Most drug-related AEs were Grade 1-2. Grade 3-4 drug-related AEs were reported in 199 (14.9%) subjects, and 2 (0.2%) subjects reported Grade 5 events (dyspnoea and pulmonary fibrosis). The types of treatment-related AEs, as well as the rates of treatment-related AEs, appeared comparable to historical nivolumab monotherapy rates. All treatment-related AEs, except for 1 Grade 4 myocarditis (240 mg relatlimab/240 mg nivolumab Q2W), as well as 1 Grade 4 potential drug-induced liver injury, 1 Grade 5 dyspnea, and 1 Grade 3 pneumonitis (all at a dose level of 80 mg relatlimab/240 mg nivolumab Q2W), were reversible and manageable by withholding study treatment administration, providing standard medical care, and/or following immune-related AE algorithms. Safety results to date indicate that sequential, coadministration, and the FDC of relatlimab + nivolumab have similar safety profiles.

1.7 Microdialysis for cytokines

Microdialysis has been well established for 25 years as a safe, reliable way of measuring the *in vivo* concentrations of various molecules in the microenvironment of various human tissues, including the brain[30]. The probes, which can be implanted directly into the brain through minimally invasive openings into the skull, are catheters which contain small semi-permeable membranes at their tips that allow the diffusion of small molecules in and out of the probe[31]. By slowly infusing isotonic fluids in and out of the system, the levels of various solutes within the brain can be determined without the need for multiple biopsies of the area of interest. Initial work in the brain focused mostly on

neurotransmitters and on changes in their levels in specific areas of the brain in response to various stimuli. The technique of placement of these probes within the brain has been performed safely for years [32]. Due to research on the inflammatory response to trauma, there is a well-established body of literature on the practice of recovery of cytokines from the brain by dialysis, and this has recently been safely applied to brain tumor patients[33]. In this study, interferon gamma levels were undetectable after the inflammatory response to surgery had died down. These soluble factors can give a real-time accounting of the inflammatory response to an insult in the brain, giving a level of detail about reactions to immune stimuli that would be completely unattainable by other methods.

For our study, we propose the use of microdialysis to determine if checkpoint inhibitors have their desired effect in the tumor microenvironment. Presently, an anti-tumor immune response is suspected when serial MRI imaging over several months documents initial increase in tumor enhancement, signifying an inflammatory response to therapy, followed by reduced volume of enhancing tumor indicating tumor regression. Interpretation of anti-tumor response by MRI is complex because the MRI appearance of an inflammatory response to immunotherapy can appear similar to that of tumor recurrence or growth. Instead of waiting months to determine if an immune response to the tumor has occurred, we intend to use microdialysis of cytokines as a real-time test to measure immune response to a checkpoint inhibitor shortly after its administration, providing an answer within days of the first dose. Recent study in mice has shown that PD-1 blockade leads to an upregulation of interferon gamma production in circulating T lymphocytes responding to an infection[34], and this agrees with previous research that shows that interferon gamma is upregulated when PD-L1 is blocked *in vitro*[35]. Further, in recurrent glioblastoma patients, there is evidence that regulatory T cells in the systemic circulation of these patients release increasing amounts of IFNg (interferon gamma) in response to nivolumab[36]. Of note, other markers of cytotoxic activity, such as Granzyme A and Perforin 1, are much larger proteins than IFNg, and as such reliable recovery of this proteins by microdialysis is unlikely. *As such, our hypothesis is that checkpoint blockade with Nivolumab will lead to an upregulation of the inflammatory cytokine interferon gamma in the tumor microenvironment as measured by cerebral microdialysis.* After the intensive inpatient monitoring period, patients will be placed on combined therapy of BMS-986016 and Nivolumab in combination. In this way, we will be able to continue to follow immune cell populations in the blood of patients, so that we can determine if the systemic effects of checkpoint blockade differ between patients on Nivolumab versus patients on the combination of Nivolumab and BMS-986016 treatment.

1.8 Conclusion

This protocol seeks to enroll patients with recurrent glioblastoma for treatment with nivolumab and for immune monitoring with cerebral microdialysis. Currently, there is not an established standard of care for these recurrent glioblastoma patients, and so they will not be forgoing any proven therapeutic options. The study is designed primarily to discover whether interferon gamma will be produced within the brain in response to

systemic checkpoint inhibitor therapy. Patients will undergo a surgical procedure that includes brain tumor needle biopsy to confirm the diagnosis of recurrent glioblastoma. If the diagnosis of recurrent glioblastoma is confirmed by frozen section, they will proceed with the remainder of the protocol. If this diagnosis cannot be definitively confirmed on frozen section, the patient will not proceed with the placement of microdialysis catheters or lumbar drain. If the patient does have frozen section evidence of recurrent glioblastoma, 2 microdialysis catheters will be implanted, one into the tumor, and one into the surrounding brain. A lumbar intrathecal catheter will also be inserted to serial sample cerebral spinal fluid (CSF). A bone marrow examination may be performed to evaluate the baseline status of the patients' hematopoietic tissue. To allow the inflammatory response to catheter implantation to subside, we will wait two days after surgery before administering the PD-1 inhibitor, Nivolumab. We will then take samples every 6 hours from the microdialysis catheters, and daily samples from blood and cerebral spinal fluid. At the end of five days, we will remove the dialysis catheters and lumbar intrathecal catheter and resect the recurrent glioblastoma tumors. A repeat bone marrow examination may be performed to evaluate if Nivolumab causes any changes in the patients' hematopoietic tissue. Following tumor resection, the patient will receive Nivolumab and an anti-LAG-3 antibody, BMS-986016 , until tumor progression or until the patient experiences an immune mediated toxicity that prevents further treatment, as defined in Appendix A. We will analyze the cerebral spinal fluid, microdialysis fluid and blood for immune markers by proteomic analysis. We will measure changes in immune markers in the various compartments (brain tumor, brain, blood, CSF and potentially bone marrow) between before and after their first dose of nivolumab and determine the relative immune effects of Nivolumab in these compartments. Recent data in melanoma patients suggests that after a month of treatment, changes in histological markers of immune response such as cluster of differentiation 8 (CD8), CD4, CD3, PD-1, PD-L1 and lymphocyte activation gene 3 (LAG3) may be predictive of response to PD-1 inhibitors[37]. As such, if we are unable to establish interferon gamma as a suitable marker of immune response, we will evaluate pathological response as our primary outcome measure.

The relationship between markers of inflammation in the interstitial fluid, cerebral spinal fluid and serum have been investigated by the neuroimmunology community for years, but there are many answers left to be unlocked. This protocol aims to gather a truly unprecedented collection of data which could be used to better understand the unique immune environment of the brain as compared to the rest of the body. Potential gains could be a better understanding of how cerebral spinal fluid samples reflect changes in the immune microenvironment of the brain – is there a reliable relationship between cytokine levels in the interstitial fluid and cerebral spinal fluid, or are they completely different and unrelated? Are there other proteins that are better predictors of the nature of the immune populations in the brain? Are there serum markers that better predict intracranial inflammation than CSF markers do? Such findings could be applied to other neuroinflammatory disorders, such as multiple sclerosis or cryptococcal meningitis. The technique of microdialysis for cytokines, using proteomic analysis of the dialysates, which has not been done, could be applied to other disorders as well. For all of these reasons, while we feel that this protocol may provide critical information in the treatment

of brain tumors, we also feel that the study has the potential to create generalizable knowledge that will have a high impact in the scientific community. The National Institutes of Health Clinical Center is the only institution that is perfectly positioned to perform a trial such as this with the potential to truly revolutionize how we view inflammation in the brain.

2.0 Study Objectives

2.1 Primary Goal

To determine the proportion of patients who have a measurable increase of interferon gamma levels in the brain tumor tissue after their first dose of Nivolumab as compared to the pre-treatment baseline as measured by cerebral microdialysis, to evaluate the safety of using brain tumor microdialysis to monitor response to immune modulators in patients with recurrent glioblastoma and to evaluate the safety of the combination of Nivolumab and BMS-986016 antibody treatment.

2.2 Exploratory Goals

1) To determine the change in interferon gamma production within the tumor microenvironment and in the rest of the body from before and after therapy with the immune checkpoint inhibitor, nivolumab;
2) To evaluate the pathological response of the immune microenvironment of brain tumor tissue to the first dose of Nivolumab;
3) To evaluate the clinical response (progression free survival, overall survival) of recurrent glioblastoma patients to treatment with BMS-986016 and Nivolumab;
4) To describe the difference in survival between responders and non-responders;
5) To examine the differences in the immune cells and secreted factors of the tumor environment as compared to the immune cells and secreted factors of the cerebral spinal fluid, blood and potentially bone marrow in response to this treatment;

3.0 Subjects

3.1 Description of study populations

- The study population will consist of patients, 18 or older, with first recurrence of their previously treated glioblastoma.
- Accrual ceiling will be 25
- Target number of completed patients will be 10
- Withdrawals who do not complete the initial study procedures, specifically, microdialysis monitoring period and resection portion of the study, will be replaced up to the accrual ceiling of 25 patients

- NIH employees may participate. NIH employees who are subordinates/relatives/co-workers of the investigators can be considered for inclusion in the trial on a case by case basis

3.2 Inclusion Criteria

To be eligible for entry into the study, a candidate must meet all the following criteria:

1. Be 18years of age or older.
2. Have recurrent glioblastoma that is amenable to surgical resection.
3. Agree to undergo brain surgery.
4. Are eligible for 03-N-0164 "Evaluation and Treatment of Neurosurgical Disorders" protocol
5. Willing and able to appoint a durable power of attorney.
6. Are willing to use an effective method of contraception during the clinical study as defined on the consent and for 24 weeks (for women) or 33 weeks (for men) after the last dose of the study drug.

3.3 Exclusion Criteria

Candidates will be excluded if they:

1. Have a bleeding disorder that cannot be corrected before invasive testing or surgery, or other medical conditions that would make surgery unsafe, such as lung or cardiac disease that would render them unable to tolerate the risk of general anesthesia, or severe immunodeficiency.
2. Has a known additional malignancy that is progressing or requires active treatment within 3 years of registration. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.
3. Are pregnant or breastfeeding
4. Cannot have an MRI scan.
5. Are claustrophobic
6. Are not able to lie on their back for up to 60 minutes
7. Have primary CNS lymphoma.
8. Has received systemic immunosuppressive treatments, aside from systemic corticosteroids (such as methotrexate, chloroquine, azathioprine, etc) within six months of registration.
9. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment
10. Have a significant cardiac history, such as 2 or more MIs OR 2 or more coronary revascularization procedures.

11. Have abnormal findings on ECG such as prolonged QT interval, T-wave abnormalities or arrhythmia. Abnormal findings on ECG will prompt an evaluation by a cardiologist prior to enrollment in the study
12. Are currently undergoing treatment with another therapeutic agent for glioblastoma
13. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).
14. Have an ejection fraction <50% on screening echocardiogram
15. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies) at the time of enrollment.
16. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected) at the time of enrollment.
17. Have an active infection that requires systemic antibacterial, antiviral or antifungal therapy < 7 days prior to initiation of study drug therapy
18. Have a history of transfer of autologous or allogeneic T cells
19. Have a history of solid organ or tissue transplants
20. Have cardiac Troponin T or I > 2x the institutional upper limit of normal at screening
21. At the time of enrollment, lack of consent capacity due to cognitive impairment that would make them incapable of understanding the explanation of the procedures in this study. Cognitive capacity to consent will be determined at the time of enrollment. Patients with mental disorders or those patients who are cognitively impaired yet still retain consent capacity will not be excluded.
22. Cannot speak English or Spanish fluently
23. Patients that require dexamethasone > 4 mg/ day or equivalent of steroids

4.0 Study Design and Methods

4.1 Study Overview

The NINDS neurology and neurosurgery staff, in collaboration with the Neuro-Oncology Branch (NOB) in the National Cancer Institute, will evaluate patients with recurrent glioblastoma for this study. Those patients for whom standard of care would involve surgical resection of their radiographic recurrence will be eligible for the trial. Enrolled patients will undergo MRI before undergoing surgery. Stereotactic biopsy will be performed to confirm the recurrence. If the frozen section from the biopsy does not confirm recurrent tumor, the patient will not undergo further study procedures and will be replaced. If the diagnosis of recurrent tumor is confirmed, microdialysis catheters will be placed in the tumor and in the peritumoral area. A lumbar drain will also be placed at the time of surgery, and the patient will be admitted to the intensive care unit. Samples will be collected from the microdialysis catheters every 6 hours. Collection of cerebral spinal fluid and blood samples will be done daily. Two days after surgery, Nivolumab will be administered. Samples will be collected daily from blood and cerebral spinal fluid and

every 6 hours from microdialysis catheters until 5 days after Nivolumab administration. The patients will then have their tumors resected, and extracted tumor tissue will be processed for immune cell infiltration. Patients will then receive outpatient treatment with nivolumab and BMS-986016 every two weeks (+/- 2 days) and outpatient clinical monitoring with MRI every four weeks until tumor recurrence.

Relationship to other protocols:

Patients being followed by the NOB may also be enrolled in their natural history protocol. All patients at the NIH Clinical Center site will be enrolled in the **03-N-0164:** Evaluation and Treatment of Neurosurgical Disorders protocol for tissue banking related to surgical resection.

4.2 Recruitment

Subjects at the NIH Clinical Center site will be referred by the Neuro-Oncology Branch, self-referred through the Patient Recruitment Public Liaison (PRPL) office, recruited from patients already participating in 03-N-0164, or referred through community physicians. Advertisements will be pre-approved by the IRB. Subjects who are interested in participating in this study will send medical records for review to determine whether they meet the criteria for enrollment. There will be no direct solicitation or recruitment of NIH employees.

The direct link to the study information on clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT03493932) and/or the direct link to study information on the NINDS "Clinical Trials in the Spotlight" public web page [*the link to the direct study information on Spotlight will go here once approved by the IRB]* will be posted periodically on the official NINDS Twitter and Facebook accounts. The NINDS "Clinical Trials in the Spotlight" public web page for the trial will contain the same language as the IRB approved flyer. IRB approved ads and/or flyers (Appendix F) may also be posted on the official NINDS Twitter and Facebook Accounts.

Flyers may be posted electronically on websites such as NIH or NCI websites, advocacy support group websites such as the American Brain Tumor Association or National Brain Tumor Alliance, publications' websites including the Washington Post.

During prescreening, physician investigators on this study will review medical records to determine if patients likely have recurrent glioblastoma. If a potential subject does not meet the inclusion criteria for the study, or if they choose not to enroll, their records will be destroyed or returned at the person's request. Expected accrual rate is 1-2 patients per month.

4.3 Screening Methods

After a patient has signed consent for this protocol, screening will occur, during which patients will have a history and physical exam that may identify unsuspected exclusion criteria. The patient will undergo blood tests, including a troponin, an EKG, a chest X-ray

and an echocardiogram. Study investigators will again review records to attempt to identify exclusion criteria. Screening will also include a blood or urine pregnancy test for women of childbearing age, as pregnant women cannot be enrolled in the protocol. After screening has completed, the patient will be fully enrolled in the trial.

4.4 Study Design

This protocol is an open label, non-blinded single armed pilot study of the use of nivolumab and BMS-986016 in recurrent glioblastoma patients.

4.5 Study Procedures

4.5.1 Summary of Research Procedures (Study Phase 1)

Patients will receive pre-operative structural MRI and pre-operative blood work. Pre-operative imaging and blood work will occur between the time of enrollment and the surgical procedure. Clinical care considerations and the availability of the operating room schedule will determine the timing and acuity of these tests, and these tests will occur between several days to 2 weeks before surgery.

At the time of surgery, adults will be admitted to the Neuroscience Patient Care Unit (NPCU). The patient will be asked to sign a standard surgical consent form that covers the tumor biopsy, microdialysis probe placement, and lumbar drain placement. These surgical procedures will be considered the beginning of Phase 1 of the trial for safety monitoring purposes. For the research surgical procedure, on study day 1, the stereotactic biopsy will be performed using frameless stereotaxy. A frozen section will be sent from the biopsy tissue. If the biopsy confirms recurrent tumor, the patient will continue with study procedures. If the biopsy is not consistent with recurrent tumor, the patient will not undergo further study procedures and will be replaced. The burrholes for the microdialysis catheters will be placed using stereotactic guidance in a similar fashion to the biopsy procedure. Lumbar drain placement will also be performed in this same surgical procedure. While the patient is under anesthesia, a hematology fellow may perform a bone marrow examination. Following biopsy and microdialysis catheter implantation, subjects will stay in the ICU for 24 hours, and then be moved to a private inpatient room with a one-on-one sitter until the time of resection, 7 days after the initial biopsy. The first dose of Nivolumab will be given at 48 hours after the biopsy on study day 3. The lumbar drain catheters will remain clamped in between CSF sampling to allow the patients to be mobile during their inpatient stay. A stereotactic CT scan will be performed after the biopsy and microdialysis catheter placement to verify accurate placement and assess for procedural complications. Phase 1 will end at the start of surgical resection of the tumor, and clinical care will then commence as outlined below. Primary outcome measures will be assessed at the conclusion of enrollment, as all samples will be sent for cytokine testing at that time. All patients will come for a safety monitoring visit 2 weeks after their second surgery (+/-1 week) for safety evaluation. All patients will continue with routine examinations and Nivolumab and BMS-986016

therapy every 2 weeks, starting on study Day 17 (+/- 2 days) until recurrence or a dose limiting toxicity is experienced with appropriate imaging.

4.5.2 Summary of Clinical Care Procedures and follow up (Study Phase 2):

For safety monitoring purposes, Phase 2 will commence at the beginning of surgical resection of the tumor, as part of the patient's clinical care. Surgical resection performed per standard of care procedure. Apart from surgery, as noted in the introduction, there is no current established standard of care treatment for recurrent glioblastoma patients. While oncologists have the option of using BCNU impregnated carmustine wafers or Bevacizumab, these treatments have been shown to have questionable benefit in glioblastoma patients. In short, a craniotomy will be planned and performed depending on the location of the recurrent tumor and the previous incisions. A maximal safe resection of the recurrent tumor will be performed utilizing stereotaxis, functional monitoring, and intra-operative imaging as needed. After the surgical tumor resection and removal of the dialysis catheters and lumbar drain, the patient will be admitted to the ICU until stable, and then they will be transferred to the Patient Care unit for routine post-operative care. The decision to transfer patients from the ICU to the NPCU and decision regarding discharge from the hospital following surgery will depend on clinical care considerations only and are dependent on the individual patient. Patients will receive post-operative structural MRI, and may receive a CT as clinically indicated. Upon discharge from the hospital, a summary letter will be sent to the patient's physician of record, describing the care rendered. All patients will be seen in the Outpatient Neuroscience Clinic 2 weeks (+/- 1 week) after their surgery and will undergo history, physical and neurological examination at that time. This visit is for post-operative care and is separate from the scheduled Day 17 (+/-2) visit.

4.5.3 History, Physical Examination, and Neurological Examination (Research and Clinical Care)

A credentialed doctor, physician assistant or nurse practitioner who is capable of making an independent determination of the patient's suitability for this trial will take a medical history and perform a physical examination. This clinician will have to be knowledgeable about the study as a whole, particularly the risks of the study interventions and eligibility criteria. This physical examination is for purposes of clinical care only and does not replace any examination the patient may receive from his/her own physicians. For patients who have been referred to the Surgical Neurology Branch as a surgical candidate, this history and physical will be deemed sufficient as part of the evaluation for participation in the trial. If the patient in question is a patient currently being followed by the Surgical Neurology Branch, such that they have not been specifically referred to the Surgical Neurology Branch for surgical resection prior to being identified as a trial participant, the patient's records will be sent to an outside independent neurosurgeon to determine suitability of the patient for repeat surgical resection. The history and physical will be repeated at each visit for Nivolumab and BMS-986016 infusion and monitoring, every two weeks (+/- 2 days).

4.5.4 Pregnancy testing (Research)

Pregnant women may not be enrolled in this protocol, and pregnancy will be determined through serum or urine testing during screening. Subsequent pregnancy testing will be performed prior to each scheduled MRI (monthly, during follow-up). Women of childbearing age will have to agree to use an effective method of contraception for the duration of their participation in the protocol and for 24 weeks post last treatment of Nivolumab. Men will have to agree to use an effective method of contraception for the duration of their participation in the protocol and for 33 weeks post last treatment of Nivolumab. For the purposes of this study, effective methods of contraception will be considered to be: hormonal contraception (birth control pills, injected hormones or vaginal ring); intrauterine device; barrier methods (condom or diaphragm) used with spermicide; surgical sterilization (hysterectomy, tubal ligation, or vasectomy).

4.5.5 Blood drawing (Research and Clinical Care)

Blood will be drawn through a needle in the patient's arm. For adults, blood tests will be drawn prior to MRI to evaluate kidney function (if required per MRI contrast requirements) and before surgery to evaluate serum chemistry, including markers of liver functioning, hematology and coagulation. Up to 35ml of blood will be withdrawn for research purposes at the time of study enrollment. Up to 35ml of blood will also be drawn daily during the immune monitoring period for research including pharmacokinetic analysis after checkpoint inhibition to determine the expression of inflammatory markers and cell content of the blood at these time points. On Day 3, additional samples of up to 10 ml of blood (total on Day 3 up to 45ml) will be drawn. Up to 5ml of blood will be withdrawn before, and 6 hours +/- 2 hours after the completion of the nivolumab dose for pharmacokinetic analysis. Up to 35ml of blood will also be drawn at every 2 week (+/- 2 days) visit for Nivolumab infusion. We will not exceed 550ml in any 8-week period, consistent with existing IRB limits.

4.5.6 Electrocardiography (ECG/EKG)

We will perform an ECG during the initial enrollment into the trial and at each 2-week (+/- 2 days) follow-up appointment to detect signs of cardiomyopathy. Prolonged QT interval, arrhythmia, T-wave abnormalities or any other concerning ECG findings will prompt a consultation to cardiology to rule out pre-existing cardiac pathology prior to dosing of the study drugs.

4.5.7 Echocardiogram

The echocardiogram uses sound waves to look at the patient's heart. A standard echocardiogram will be performed at the initial enrollment into the trial.

4.5.8 Chest X-ray (Clinical care)

All patients will have a chest x-ray as part of their routine clinical evaluation prior to enrollment in the trial. The chest x-ray is being performed as routine clinical care as all

patients who undergo neurosurgery at the NIH Clinical Center are required to have a chest x-ray by anesthesia.

4.5.9 Structural MRI (Magnetic Resonance Imaging) (clinical care and research)

MRI evaluation is standard of care for patients with brain tumors. During the initial evaluation, patients may have imaging from an outside institution that can provide diagnostic information. However, prior to surgery, patients under this protocol will need to have a recent structural (no older than two weeks prior to biopsy/microdialysis placment) MRI obtained. This MRI is for standard clinical care. After the standard of care surgical resection, another MRI will be performed. This MRI is also for standard clinical care. MRI will also be repeated every 4 weeks during the treatment period with Nivolumab and BMS 986016 or earlier as indicated due to change in symptom burden. These MRIs are for research purposes. MRI uses a strong magnetic field and radio waves to take pictures of the brain. The MRI scanner is a metal cylinder surrounded by a strong magnetic field. During the MRI, the patient will lie on a table that can slide in and out of the cylinder. The patient will be in the scanner about 60 minutes. The patient may be asked to lie still for up to 15 minutes at a time. While in the scanner, the patient will hear loud knocking noises, and will be fitted with earplugs or earmuffs to muffle the sound. The patient will be able to communicate with the MRI staff at all times during the scan, and may ask to be moved out of the machine at any time.

MRI scans may include intravenous administration of an FDA-approved, macrocyclic, gadolinium-based MRI contrast agent. Unless otherwise specified in the protocol, contrast agents will be used at FDA-approved doses. NIH Clinical Center Radiology and Imaging Sciences guidelines for gadolinium administration will be followed. Under certain circumstances, participants in this study may receive a linear chelate. Examples of situations in which this may occur include the following: (1) prior sensitivity to all macrocyclic agents; (2) participation in a longitudinal study in which a linear chelate was previously given, there is a clear advantage to maintaining the same gadolinium protocol, and T1 signal change in the basal ganglia or dentate nuclei was not observed; or (3) after consultation with, and approval by, a radiologist credentialed at the NIH Clinical Center. A list of the instances in which a linear chelate was administered, along with the justification for its administration, will be provided to the IRB at the time of continuing review.

To assess white matter tracts, DTI will be performed in all patients. Data are acquired with a single-shot spin-echo echo-planar imaging sequence that utilizes standard acquisition and reconstruction methods. The data are acquired with isotropic voxels dimensions of ~2 mm, full k-space coverage, minimal TE based on the b-value, minimal TR based on required imaging time to cover the whole brain, and acceleration from parallel imaging. A custom set of diffusion directions and weightings are acquired with maximal b-value= 1100 s/mm^2. The total scan time for the DTI acquisition is approximately 20 minutes.

4.5.10 Research surgical procedure (Research)

Following preoperative evaluation, patients will be admitted (Study Day 0) and the study will begin with a stereotactic biopsy of the patient's recurrent lesion and the insertion of two small microdialysis catheters into the brain. For safety monitoring purposes, this will be the beginning of Phase 1 of the study. One microdialysis catheter will be placed in the tumor and one into the peritumoral area. On the day of surgery, general anesthesia will be administered in the operating suite.

4.5.10.1 Lumbar drain placement (Research)

A lumbar drain will be placed in each patient in the standard fashion. The patient will be placed on their left or right side, depending on what makes the most sense for the subsequent craniotomy. The patient's knees will be pulled towards their chest. The touhy needle supplied with the lumbar drain catheter will be inserted with the bevel parallel to the ligaments at a cranial/caudal level most consistent with L4/5, using the patient's posterior superior iliac crests as landmarks. The needle will be guided towards the umbilicus until it is in the intrathecal space, and the bevel will be turned cranially. The lumbar drain will then be inserted through the needle into the intrathecal space. The needle will then be removed, CSF flow will be confirmed, and the catheter will be attached, sterilely, to the CSF drainage bag and clamped. In the event that the lumbar drain fails during the microdialysis monitoring period, the lumbar drain will be removed. The patient will be offered the option of replacement of the lumbar drain on the floor or fluoroscopy suite (as medically indicated), or lumbar puncture, below. Placement of the lumbar drain on the floor would be performed as above.

4.5.10.2 Lumbar puncture (Research)

In the event that the lumbar drain fails and the patient opts against replacement of the lumbar drain on the floor, a lumbar puncture will be performed in the operating room on Day 8 for research purposes. This will be performed under general anesthesia. For the lumbar puncture, the patient will lie on their side, curled up with their knees at their chest, or sitting upright. Their lower back will be cleaned. A needle will be inserted through the numbed skin and into the space between the bones in their back. Up to 15 ml of cerebrospinal fluid (CSF) will be removed for research purposes, beyond what CSF is lost during usual performance of the procedure. After the fluid is collected, the patient will continue with surgical resection as indicated.

4.5.10.3 Bone Marrow Examination (Research)

For the bone marrow examination, aspirate and core biopsy may be collected according to conventional clinical technique from the hip of the patient. If the procedure is performed, it will be done by a clinical provider credentialed to perform this procedure, and is typically performed at the end of the research surgical procedure while the patient is under anesthesia. The decision to perform this examination is at the discretion of the primary investigator.

4.5.10.4 Stereotactic biopsy (Research)

Patients will undergo placement of a surgical head frame and will be registered into our neuronavigation system. A plan will be made on the neuronavigation for the location of the biopsy and the targets for placement of the microdialysis catheters. The biopsy and catheter sites as planned above will be prepped and draped in the usual sterile fashion. First, at the biopsy site, a small stab incision will be made, and the pneumatic drill will be used to make a small burr hole in the skull in the trajectory of the needle biopsy. The dura will be incised, the pial surface of the brain will be coagulated, and we will then open the pia. The needle will then be passed along the trajectory to the target using the neuronavigation system. Adequate biopsies will be taken to confirm the diagnosis of recurrence. This confirmation will be done by frozen section. There will be at least 4 samples taken, but there may be as many as 20, from different sites, if the diagnosis is hard to confirm. If a diagnosis of recurrent glioblastoma cannot be confirmed by the frozen section, the patient will not proceed with the rest of the study procedures. Once adequate tissue has been removed, the needle will be removed.

4.5.10.5 Microdialyis catheter placement (Research)

After the brain biopsy is performed confirming recurrent glioblastoma, the neuronavigation system will be utilized to plan a needle trajectory from two skull burr holes to the dialysis probe targets. The MD 71 catheter we will implant has one inlet and one outlet tube which is designed for sampling only. The surgeon's discretion will be used to pick solid, contrast-enhancing portions of the lesion that appear to best represent the most pathological portion of the tumor for microdialysis catheter placement. The surgeon's discretion will also be used to pick a second site that does not appear to be contrast-enhancing tumor for the peritumoral catheter. A stab incision on the scalp will be made at the site of each planned burr hole, one planned to go into the contrast-enhancing tumor, and one into the peri-tumoral area, at least 10mm away from the contrast enhancing margin of the tumor, using neuronavigation system guidance as above. This will be done using the surgeon's discretion. We will assess the peritumoral catheter target using the following criteria:

1) A lack of contrast-enhancement as noted on the neuronavigation system
2) The closest adequate area to the tumor site, as assessed by the surgeon
3) Adequate brain tissue surrounding the placement site to allow the catheter to sit in the tissue undisturbed, as assessed by the surgeon
4) A safe path to the insertion site, as assessed by the surgeon

Once the dura and pial surface are pierced, as above, the catheter will be passed along the established trajectory into the brain tissue. A guide needle may be used to place the microdialysis catheter. The part of the catheter outside the skull will be tunneled under the skin to a second stab incision, where it will exit the scalp. The ends of the tubing connected to the cannulas will be sterilely sealed and secured. The scalp wound will be closed, and the head frame removed while anesthetized. Patients will be awakened from anesthesia and returned to the Intensive Care Unit for immediate postoperative care. The dialysis machines will be attached to each catheter in the ICU.

4.5.11 Computer-assisted Tomography (CT) Scan (Research)

This research study involves exposure to radiation from a CT scan of the head following biopsy/microdialysis catheter placement surgery to evaluate for the development of a blood clot and accurate placement of the microdialysis catheters.

4.5.12 Microdialysis sample retrieval (Research)

Upon arrival in the intensive care unit, the MD 71 High Cut off brain microdialysis catheters will be connected to a portable syringe pump to perfuse artificial CSF (Perfusion Fluid CNS, ref. no. P000151, CMA, Solna, Sweden) with 3.5% human serum albumin at a rate of 0.3 µl/min for approximately 7 days. The inlet tubing of the catheter will be connected to a CMA 107 Microdialysis Pump (ref. no. P000127, a, Solna Sweden). From the immediate post-operative period until 24 hours post-microdialysis implantation, the patients will be monitored in the ICU and then moved to an inpatient floor with a one-on-one sitter until study day 8 due to the presence of the microdialysis catheters. Microdialysis samples will be removed from the microdialysis system every 6 hours +/- 2 hours using the collection system of the microdialysis system. In the event that the microdialysis catheter must be removed prior to the completion of the microdialysis monitoring period for reasons, including, but not limited to, microdialysis catheter failure, complication requiring early removal of microdialysis catheters, etc, the catheter will be removed on the unit and the patient will continue with into Phase 2 of the trial with surgical resection as indicated.

4.5.13 CSF Sample retrieval (Research)

CSF will be taken daily while the subject is in the ICU and during the patient's inpatient stay. The lumbar drain will remain clamped until the time of sampling. At that time, the first 5ml will be drained off to allow the clearance of debris and sediment, and then up to 15ml of CSF will be collected for studies and stored at -80 degrees C. On Day 3, additional samples of up to 10ml of CSF will be drawn (total for Day 3 up to 25ml of CSF). Up to 5ml will be withdrawn before, and 6 hours +/- 2 hours after the completion of the nivolumab dose for pharmacokinetic analysis. In the event that the lumbar drain fails and is not replaced, CSF collection will cease until the lumbar puncture performed in the operating room on Day 8. Up to 15ml will be withdrawn in the operating room during the lumbar puncture.

4.5.14 Nivolumab (Research)

Nivolumab is a programmed death receptor-1 (PD-1) blocking antibody. 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. The FDA has recently approved Nivolumab for the treatment of patients with recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN) with disease progression on or after platinum-based therapy; and it was also approved for treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease

progression within 12 months of neoadjuvant or adjuvant treatment with a platinum-containing chemotherapy. 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. This drug will be provided as part of a drug-only sponsorship from Bristol-Myers Squibb. It will be given on study day 3 at a dose of 240mg given intravenously over 30 minutes. It will be given subsequently as an intravenous infusion over 30 minutes at a dosage of 240mg every 2 weeks together with BMS 986016, starting on Day 17 (+/- 2 days), as outlined in Section 1.6.4, above. It is produced by Bristol-Myers Squibb, New York, NY. The Investigator Brochure is listed as Appendix B. Please see section 4.5.19 for definition of duration of therapy.

4.5.15 Anti-LAG-3 antibody, BMS-986016 (Research)

BMS-986016 is a Lymphocyte Activation Gene-3 (LAG-3) blocking antibody which is undergoing study both as monotherapy and as combination therapy with Nivolumab for the treatment of advanced cancers. This drug will be provided as part of a drug only sponsorship from Bristol-Myers Squibb. It will be given beginning on study Day 17 (+/- 2 days). It will be given as an intravenous infusion over 60 minutes at a dosage of 80mg every 2 weeks (+/- 2 days). It is produced by Bristol-Myers Squibb, New York, NY. The Investigator Brochure is listed as Appendix C. The patients will have BMS-986016 infusions every 2 weeks (+/- 2 days) with the Nivolumab until progression. Please see section 4.5.19, below, for discussion of duration of therapy.

4.5.16 Surgical Treatment and Post-operative care (Clinical Care)

After the conclusion of microdialysis monitoring, patients will undergo resection of their brain tumor as per protocol 03-N-0164. The beginning of this surgery will be noted as the start of Phase 2 of the study for safety monitoring purposes. The type of surgery that will be performed will be decided upon by the neurosurgeon and will be discussed with the patient before surgery.
When deemed necessary, as per standard clinical practice, a surgical procedure will be performed with intraoperative motor, sensory and speech mapping. The decision to perform a surgical procedure in an awake vs anesthetized manner will be determined based on standard clinical practice. Generally, awake surgery is employed in the case of language mapping, and in some cases for motor mapping, whereas intraoperative monitoring with anesthesia may be used for motor mapping. This surgery will be done in accordance with standard of care. The patients will receive a post-operative MRI, and will be watched in the Intensive Care Unit, as would be our typical practice, until the patient is stable for transfer to the Neurosurgical floor at the discretion of the treating physicians. When the patient is stable for discharge from the hospital, they will be discharged home or to a suitable rehabilitation facility, as indicated.

4.5.17 Repeat Bone Marrow Examination (Research)

For the bone marrow examination, aspirate and core biopsy may be collected according to conventional clinical technique from the hip of the patient. If the procedure is performed, it will be done by a clinical provider credentialed to perform this procedure, and typically is performed at the end of the research surgical procedure while the patient is under anesthesia.

4.5.18 Surgical specimens (Research and Clinical Care)

A portion of the brain tissue specimen will remain after the neuropathologist removes the amount that is required to make a pathologic diagnosis at both biopsy and resection. This remaining tissue will be used for research purposes, consistent with NIH guidelines under protocol 03-N-0164. Tissue obtained through this protocol will be evaluated for molecular, cellular, and genetic markers of tumor tissue and normal tissue. Specimens will be evaluated for inflammatory markers including cytokines, interleukins, TNF-a, T cells, microglia, and reactive astrocytes. Remaining tissue will be frozen and stored for use in brain research studies in the future either at NIH or at other institutions. We will obtain consent from patients to use their specimens in future studies and at other collaborating institutions.

Brain specimens stored in the tissue bank of the Surgical Neurology Branch, NINDS, will remain frozen until they are used. A biologist in the Surgical Neurology Branch will track brain specimens.

The banked and processed research specimens are coded and stored without personal identifiers. The specimens and data from this research will remain stored after completion of the protocol. Because research specimen testing does not affect the care of the patient, the patient will not be notified of the results of his/her research tests, nor will the results appear in the medical record or be given to the referring physician.

4.5.19 Follow-up (Research)

Upon discharge from the hospital, a summary letter will be sent to the patient's physician of record, describing the care rendered. Dosing of study medications will commence on Day 17 (+/- 2 days). Safety monitoring for infusions is discussed in section 8.2, below. Additionally, all patients will be seen in the Outpatient Neuroscience Clinic 2 weeks (+/- 1 week) after their surgery. This visit requirement is separate from the Day 17 (+/- 2 days) study visit. If appropriate, this post-operative care visit will be conducted in the day hospital, during the Day 17 (+/- 2 days) study visit. It may also occur on 7SW, if the patient remains admitted in the hospital, post-operatively. The patients will continue to receive Nivolumab, as above, at a dose of 240mg IV every two weeks and BMS-986016 antibody at a dose of 80mg IV every two weeks until progression. The first dose of both study medications will begin on study Day 17 (+/-2 days). Details of the infusion can be found in section 1.6.4. Between study visits, no specific precautions are needed. Patients will be reminded to call us with any clinical concerns regarding potential adverse events, including, but not limited to GI concerns, neurological compromise, signs of adrenal

insufficiency, skin lesions, hypophisitis, respiratory concerns, cardiovascular compromise, renal insufficiency or liver failure. Detailed safety monitoring procedures are outlined in section 8, below. Section 8.6 contains the list of toxicities which will prompt automatic discontinuation of the study drugs. Additionally, we will follow the I-O treatment algorithm for Nivolumab in Appendix B, below. Steroids will be used for symptomatic management as needed, per the discretion of the treating physician. Steroid use will not be a criterion for removal from the study.

Prior to documenting removal from protocol therapy, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.
In the absence of treatment delays due to adverse event(s), treatment may continue as specified in the above treatment modality sections or until one of the following criteria from section 8.6, below, occurs.

Subjects initially meeting radiologic criteria for disease progression may continue receiving study medication until confirmation of progression. Progression will be defined by Response Assessment in Neuro-Oncology Criteria (RANO) criteria, as it has been adopted for immunotherapy trials[38]. The initial radiological suspicion of progression will be raised when:
- ≥ 25% ↑ sum of biperpendicular diameters of enhancing disease (new lesions will be added to the sum of the diameter of enhancing disease during the treatment period and will not automatically be assumed to be progression).

This continuation of therapy can continue for a total of 12 weeks with MRIs performed every 4 weeks, as per protocol. iRANO criteria has suggested that this treatment extension only be implemented during the first 6 months of therapy, however there is not yet enough data about treatment of recurrent glioblastoma patients with checkpoint inhibition to be sure of the time course of pseudoprogression. As such, this additional observation period will be implemented at any time while patients are on study medications. Patients will only be eligible to continue therapy in the face of MRI evidence of potential progression if the patient is believed to be demonstrating clinical benefit as deemed by the investigator, the patient is tolerating the study medications and the patient has not significantly declined, neurologically. Of note, if the patient's decline reverses with corticosteroids or surgical sampling of the patient's mass demonstrates treatment effect only, the patient may continue on therapy or resume therapy.

Subjects with confirmed radiologic progression (approximately 12 weeks after initially assessed progression) will discontinue study medication and enter the survival follow up phase of the study. If progression is confirmed, then the date of disease progression will be the first date the subject met the criteria for progression.

To determine clinical progression, subjects with neurological decline will receive a clinical MRI to evaluate for progression by imaging (standard of care).

During Phase 2, in any patient with significant neurologic decline where disease progression cannot be differentiated from pseudoprogression, and/or in n those cases in which radiologic progression cannot be differentiated from pseudoprogression and it is the investigator's opinion that a surgical biopsy or resection to obtain tumor tissue for histopathology is in the subject's best interests, a surgical resection may be performed following consultation with the medical monitor and outside consultant neurosurgeon. If tumor pathology confirms progression, then the subject will be discontinued from study medication per protocol discontinuation criteria and the date of progression will be the day that it was first suspected. If the patient's decline reverses with corticosteroids and/or tumor pathology reveals treatment-related changes and does not confirm disease progression, we will discuss resumption of therapy with our Safety Monitoring Committee (for patients enrolled at NIH) and the Data and Safety Monitoring Board (for patients enrolled at the Emory Site). The subject will then continue all on-treatment tumor assessments as per the treatment schedule.

4.5.20 Other treatments for glioblastoma and concurrent medications (Clinical care)

There is no accepted standard of care treatment that patients will be forgoing during the study period. Steroid use will be permitted for symptomatic management while the patient remains on study, if indicated, at the discretion of the treating physicians in accordance with the I-O AE Algorithms from Appendices B and C; Investigator Brochures for Nivolumab (BMS-936558) and Relatlimab (BMS-986016). Any concerns regarding treatment of Nivolumab or BMS-986016 side effects will be referred to the SMC (for patients enrolled at NIH) and DSMB (for patients enrolled at the Emory Site).. Patients will not receive any other experimental or off-label treatment while on the study. As all patients will have undergone standard of care radiation at the time of admission to the study, and additional radiation is not a standard treatment for recurrent glioblastoma, no additional radiation will be given to patients while on the study. Patients will be evaluated for additional radiation or other treatments at the time of progression. If the patient is lost to follow-up prior to documented progression or death, patient will be censored as of the date of the last adequate evaluation (MRI and exam). If patient dies prior to documentation of progression, progression will be defined as the date of death, if patient has been attending regularly scheduled follow-ups.

4.6 Emory University Hospital Site

For all patients enrolled at Emory University Hospital, all procedures and follow-up will be as above, but they will be carried out in the equivalent units at Emory University Hospital. The activities performed at Emory University Hospital will fall under the oversight of the Emory University IRB.

4.7 End of participation

Active participation in this protocol and treatment with BMS-986016 and nivolumab will end upon:

- clinical progression (neurological decline followed by confirmation of progression by imaging or pathological diagnosis) or
- confirmed radiological progression and investigator determination that the patient is no longer benefiting from treatment with the study treatment or
- meeting any other "Criteria for individual subject withdrawal" as listed in section 8.6

After this point, participants will be considered on survival follow-up until death. For subjects removed for clinical or radiological progression as described above, we will perform a follow-up assessment at the next scheduled imaging visit and no less than 4 weeks after the prior assessment of disease progression.

Survival Follow-up Phase

Participants who have discontinued study medication will enter the survival follow-up phase of the study after their final follow-up assessment. Participants enrolled at the NIH Clinical Center site will continue to be enrolled in protocol 03-N-0164 and data on subject progress will be shared with this protocol (NIH medical/research records). For those who decide to pursue care elsewhere and not through protocol 03-N-0164, we will perform phone calls approximately monthly to check on the status of the individual until death. We will attempt to gather Karnofsky Performance Status Rating, ongoing treatment information, follow-up any potential side effects from this study treatment, and vital status (alive/deceased).

During this phase, patients may transfer their care to an outside physician, or they may choose to enroll in further studies at the NIH Clinical Center under the care of the investigators of this protocol. If they chose to pursue their treatment outside of the NIH, all the medical care records will be transferred to the outside physician, with the subject's permission. Further consultation at the NIH under another NIH protocol will be possible by referral.

All other procedures in this protocol will be used for clinical care purposes. However, data acquired through all procedures will also be used for research purposes to investigate the relation between checkpoint inhibition and inflammatory markers in the brain, cerebral spinal fluid, blood and potential bone marrow. These additional research components are designed to provide new knowledge regarding the mechanism of action and effectiveness of such therapies in brain tumor patients.

5 Management of Data and Samples

5.1 Sample Storage

All collected research samples, including surgical tumor specimens, CSF, microdialysate, and blood will be coded and stored in secured freezers on the NIH campus. All patients enrolled in this protocol will be assigned a sequential code and all biological samples collected for this patient will be labeled with the patient's code, date of collection, type of sample, sample number, and barcode which will be linked back to relevant sample information in the study laboratory notebooks and Labmatrix. The study samples may be shared with collaborators that have a research interest in the coded samples. Only the study investigators will have access to the code key.

Loss or destruction of samples that impacts the care or safety of human subjects will be immediately reported to the IRB. Loss or destruction of samples that would not impact the care or safety of human subjects but would impact the acquisition of knowledge, will reported to the IRB within 7 days of each occurrence.

Samples collected from patients enrolled at the Emory University site will be transferred to the NIH laboratory prior to primary analysis in accordance with technology transfer and/or Sponsor's trial agreement documents.

5.2 Research Data

Research data will be coded for storage and analysis. The codes will be maintained by the Principal Investigator and the study coordinator. Research data include data from structural imaging, neurological examination, tissue analysis of surgical specimens and analysis of fluids taken for analysis. All investigators will have access to the coded data. Electronic data will be stored on a secure server and paper data will be stored in locked filing cabinets in the investigators' offices. Research data will not be used for any type of treatment and will not be placed in the patient's chart.

Data regarding clinical outcomes will be coded and stored electronically on a secure server and in paper format in locked filing cabinets in the investigators' offices.

Data collected from patients enrolled at the Emory University site will be transferred to the NIH prior to primary analysis in accordance with technology transfer and/or Sponsor's trial agreement documents.

5.3 Data and sample sharing plan

Samples and data may be shared with other NIH protocols, other investigators, or databases/repositories, under the following guidelines:

Identified data and samples may be shared with investigators on our branch protocol 03-N-0164 as participants in this protocol are also enrolled in 03-N-0164.

Data and samples may be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases. Coded data and samples will also be shared with Bristol-Myers Squibb.

Samples and data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

As no large scale genomic data will be acquired during the course of this protocol, this protocol is not subject to the NIH Genomic Data Sharing (GDS) policy.

Under the Human Data Sharing (HDS) policy, de-identified non-genomic study data will be shared with the NINDS data repository (data.ninds.nih.gov). Furthermore, applicable (e.g., generated via CRIS) identified data will be shared via BTRIS following standard Clinical Center operating procedures including BTRIS data access policies. Data to be shared include baseline characteristics as well as key study outcome variables in a tabulated format, after adequate data cleaning, processing, and quality control. Data sharing will be performed at the time of publication and/or thereafter.

Research data will also be shared with Bristol-Myers Squibb as a result of their drug-only sponsorship of this trial. Data shared with Bristol-Myers Squibb will be coded, as defined above.

Research data that will be shared with Emory University will comply with technology transfer and/or Sponsor's trial agreement documents.

6 FDA Considerations

6.1 IND Status

We have an approved IND to pursue this study, IND# 136039

6.2 Microdialysis catheter FDA status

The tubing of the catheters will be connected to a portable syringe pump to perfuse artificial CSF (Perfusion Fluid CNS, ref. no. P000151, CMA, Solna, Sweden) with 3.5%

human serum albumin at a rate of 0.3 µl/min for 7 days. The inlet tubing of the catheter will be connected to a CMA 107 Microdialysis Pump (ref. no. P000127, CMA, Solna Sweden). The catheters used will be the 71 High Cut off brain microdialysis catheter (ref. no. 8010320). This is materially very similar to the 70 brain microdialysis catheter (ref. no. P000049, CMA, Solna Sweden) which has been cleared for FDA use, and is currently being used by one of the co-investigators, Sadhana Jackson, in a trial at Johns Hopkins University, placed directly into the tumors, with no morbidity reported as of yet. The only difference between the FDA approved CMA 70 catheter and the MD 71 catheter are that the semi-permeable membrane has pores that are 100vkD in size in the MD 71 catheter, while they are 20 kD in size on the CMA 70 catheters. Otherwise, they are made of the same material, are inserted in the same way, and are the same size, so the risk of placement should be identical. Two recurrent glioblastoma patients have had the CMA 70 catheter inserted by the study PI in the setting of an NCI trial in recent weeks with no morbidity. Badie et al.[33] recently used these catheters under IRB approval without an IDE in 10 brain tumor patients undergoing resection for intracranial tumors, and no morbidity was experienced by the patients in the study.

The CMA 107 pump is materially very similar to the CMA 106 pump (Ref P000003, CMA, Solna Sweden) which has been FDA approved for use with the aforementioned MD 71 catheter. This pump was used by Badie et al. under IRB approval without an IDE [33]. The difference between the FDA approved CMA 106 pump and the CMA 107 pump is that the CMA 107 pump has variable flow rates. That said, we will be using the flow rate, 0.3 µl/min, that is used by the FDA approved CMA 106 pump. The outlet of the catheter will be attached to 106 Syringe (Ref 8010191, CMA, Solna Sweden) in accordance with its FDA approval. The CMA 107 pump is materially very similar to the CMA 106 pump (Ref P000003, CMA, Solna Sweden) which has been FDA approved for use with the aforementioned MD 71 catheter. This catheter was used by Badie et al. under IRB approval without an IDE [33].

The Perfusion Fluid CNS is FDA approved for use with the MDialysis CMA 106 pump and CMA 70 catheter. Perfusion Fluid CNS with 3.5% human serum albumin has been shown to be superior to Perfusion Fluid CNS alone with regards to fluid recovery and accuracy of cytokine sampling in the brain[39]. Our own *in vitro* laboratory testing confirmed this finding, with higher recovery of cytokines and more consistent results (unpublished data). This fluid has also been safely used in numerous studies of cerebral microdialysis for cytokines using the CMA 71 catheter without any reported morbidity[40, 41].

Given the determination by CDRH that our investigation is considered a significant risk device study, we will ensure the following:

a) The 71 High Cutoff Microdialysis Catheter (71 MD Catheter) will be labeled appropriately with “CAUTION—Investigational device. Limited by Federal (or United States) law to investigational use.”

The device status was reviewed by the FDA as part of our IND submission for the use of Nivolumab and BMS-986016 in this patient population, and the use of the catheter was approved.

7.0 Risks and Discomforts

Risks and discomforts of the surgical procedures will be discussed with the subject on an individual basis, as is the case in the clinical practice of medicine and surgery.

7.1 History, Physical Examination, and Neurological Examination

There is a minimal risk from history, physical, and neurological examination.

7.2 Pregnancy testing

There is a minimal risk from pregnancy testing.

7.3 Risks of Blood Drawing

During this study, no more research blood will be drawn beyond 4 cups over the duration of the study. The blood tests are for clinical care and research purposes. The patient may experience some discomfort at the site of needle entry, and there is a risk of a "black-and-blue" mark. There is a remote risk of fainting or local infection.

7.4 Electrocardiography (ECG/EKG)

There is minimal medical risk or discomfort from the ECG.

7.5 Chest X-ray

There is minimal medical risk or discomfort from a chest x-ray. The chest x-ray is being performed as routine clinical care as all patients who undergo neurosurgery at the NIH Clinical Center are required to have a chest x-ray by anesthesia.

7.6 Echocardiogram

There is minimal medical risk or discomfort from the echocardiogram.

7.7 Risks of Magnetic Resonance Imaging (MRI)

People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Subjects will be screened for these conditions before having any scan, and if they have any, they will not receive an MRI scan. If subjects have a question about any metal objects being present in their body, they should inform the staff. In addition, all magnetic

objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women who are able to get pregnant will have a pregnancy test done no more than 24 hours before each MRI scan. The scan will not be done if the pregnancy test is positive.

People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. If the hearing protection comes loose during the scan, the subjects will let MRI staff know right away. Subjects will notify staff of any hearing or ear problems. Subjects will be asked to complete an MRI screening form for each MRI scan. There are no known long-term risks of MRI scans.

Symptoms from the contrast infusion are usually mild and may include feeling hot, burning, or
coldness in the arm during the injection, a metallic taste, headache, allergic reactions and nausea. In an extremely small number of individuals, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. Unless specifically allowed by the protocol, participants will not receive gadolinium-based contrast agents for research purposes if they have previously had an allergic reaction to them. Individuals with a history of anaphylaxis to other agents or chronic asthma requiring treatment will not receive gadolinium under this protocol unless they have previously received gadolinium and tolerated it well. Participants will be asked about such allergic reactions and history of asthma before a contrast agent is administered.

People with kidney disease are at risk for a serious reaction to gadolinium contrast called “nephrogenic systemic fibrosis,” which has resulted in a very small number of deaths. If subjects are 60 years old or greater or have diabetes, kidney disease or liver disease, blood work to assess kidney function will be performed within 4 weeks before any MRI scan with gadolinium contrast. Participants may not receive gadolinium for a research MRI scan if kidney function is not normal. There is no evidence for the potential of gadolinium-related toxicity in people with normal kidney function. This protocol follows NIH Clinical Center guidelines for kidney-function screening related to gadolinium administration.

Most of the gadolinium contrast is eliminated in the urine. However, recent studies have found very small amounts of residual gadolinium in the body, including the brain, by imaging and at autopsy. Macrocyclic gadolinium-containing contrast agents are substantially less likely to leave gadolinium behind than linear agents. The use of macrocyclic vs. linear agents in this study is delineated in the procedures section above. There is presently no evidence that the retained gadolinium is associated with any adverse effects.

7.8 Risks of anesthesia for the research surgical procedure

The duration of anesthesia for the research surgical procedure is expected to be approximately 120 minutes. The risks of anesthesia depend on the subject's age and concurrent medical conditions. More common side effects are temporary drowsiness, nausea, and vomiting following recovery from anesthesia. Rare side effects include aspiration, extreme changes in body temperature, hypertension or hypotension, changes in heart rate or rhythm, hypoxia, and allergic reactions to the anesthetic drugs. Some of these side effects may be life-threatening or lead to life-threatening conditions such as myocardial infarction or stroke. An anesthesiologist will evaluate subjects for potential anesthesia risks prior to the procedure. To reduce the risk of aspiration, subjects will not be allowed to eat or drink for 8 hours before the procedure, and until adequate recovery from anesthesia after the procedure. Some subjects may also have an endotracheal tube inserted to reduce this risk and facilitate mechanical ventilation. Intubation is associated with a small risk of laryngospasm, bronchospasm, hypertension, damage to teeth and lips, swelling of the larynx, sore throat, and hoarseness due to injury or irritation of the larynx.

7.9 Risks of Cerebral Microdialysis catheter placement

Risks of microdialysis will be discussed with the patients, but the research literature indicates that this does not present more than minimal additional theoretical risk. Immediat theoretical risk of the surgery for placmeent would include brain hemorrahge. A recent study by Badie et al., with microdialysis catheters being implanted in the peritumoral area in brain tumor patients after resection, reported no morbidity from microdialysis catheter placement or sample collection[33]. A study of the use of the CMA 70 catheter, materially very similar to the MD 71 catheter to be used in this study, in 174 patients revealed no incidents of hemorrhage or infection attributable to the microdialysis catheters. A study of microdialysis in recurrent glioblastoma patients prior to resection of their lesion also showed that in the 8 patients that had catheters implanted, there were no observed complications[42]. A recent article used the MD 71 catheter that we plan to use in this study to investigate the cytokine changes in glioblastoma tissue and surrounding brain to radiotherapy [43]. In this study, 11 patients had biopsies, and then microdialysis catheters were placed directly into the tumor, and another catheter was placed 10mm away from the contrast-enhancing tissue. No morbidity was noted from placement of these catheters. In another study, 7 patients with malignant glioma or lymphoma had resections of their tumor, and then two MD 71 catheters placed – one at the tumor resection margin, and one 20mm away from the resection cavity[44]. No morbidity from placement of these catheters was noted in this study. The stereotactic biopsy is being done as a part of the microdialysis implantation surgery, and it carries a risk of symptomatic hemorrhage of 1%[45]. Stereotactic biopsy with a frameless neuronavigation system carries a risk of about 1% of inaccurate biopsy [46]. If an inaccurate biopsy is performed, and a diagnosis cannot be made, the patient may have to undergo a repeat stereotactic biopsy.

7.10 Risks of Lumbar Drain Placement

Lumbar drain placement during surgery presents minimal risk to the patients. Risks would include headache, cerebral spinal fluid leak, infection, epidural hematoma, and retained lumbar drain catheter. Lumbar drain placement in the ICU or inpatient unit carries the same risks, but additionally carries the risk of back pain during placement.

7.11 Risks of Lumbar Puncture

The risks of lumbar puncture under anesthesia include headache, epidural hematoma and cerebral spinal fluid leak.

7.12 Risks of Bone Marrow Examination

Although rare, there is a potential for bleeding at the site and local infection. Bleeding can be stopped by applying local pressure, and infection can be treated with antibiotics. In the long term minimal scarring may occur but in most cases the biopsy site is indistinguishable within a few months.

7.13 Risks of Computer-assisted Tomography (CT) Scan

A CT scan of the head requires that the patient lie still for a short time while images are formed. During the CT scan, the patient will be exposed to an effective dose of 0.27 rem. The chest X-ray requires that the patient sit still, briefly, while images are formed. During the chest X-ray, the patient will be exposed to an effective dose of 0.01 rem. This is below the Radiation Safety Committee guidelines of 5 rem per year. This exposure is less than a human typically is exposed to during the year, but could result in a theoretical small increase in the patient's cancer risk.

7.14 Risks of Microdialysis sample retrieval and prolonged microdialysis catheter implantation

There is minimal risk to microdialysis sample retrieval. Microdialysis catheters have been used in clinical patient care for periods of time up to 10 days without increased risk of infection[47]. Another recently completed study with the CMA 70 catheter was also just performed in recurrent glioblastoma patients[48]. In this study, 12 patients received either a biopsy or a surgical resection, and then microdialysis catheters were placed into the wall of the resection cavity or tumor tissue, if there was residual tissue. Levels of 5-FU, the expected metabolite of the initial drug, 5-FC, were measured by microdialysis for a minimum of 8 days, and these measurements continued up to 11 days. No infections (or any other morbidity) were reported in this trial. Given the evidence, we do not believe that there is an increased risk of infection for microdialysis sampling for 7 days.

7.15 Risks of CSF sample retrieval and prolonged lumbar drainage

There is minimal risk to cerebral spinal fluid retrieval from the lumbar drain. Prolonged use of a lumbar drain in a neurointensive care unit has a risk of infection, with infection rates reported at between 4.2 and 5.7%[49, 50]

7.16 Risks of Nivolumab

In clinical trials using nivolumab for other indications, the most common adverse events (graded using the Common Terminology Criteria for Adverse Events v3.0), regardless of causality, were fatigue, decreased appetite, diarrhea, nausea, cough, dyspnea, constipation, vomiting, rash, pyrexia, and headache. Common treatment-related adverse events included fatigue, rash, diarrhea, pruritus, decreased appetite, and nausea. Serious adverse effects were rare and included Grade 3 or 4 pneumonitis, sarcoiditis and elevated alanine aminotransferase levels. In approving Nivolumab for the treatment of metastatic non-small cell lung cancer, the FDA reviewed the Checkmate 057 trial[51]. Of the 582 patients randomized in the CM057 study, 287 received Nivolumab. The most common adverse events, occurring in some grade in at least 10% of patients were: 30% of patients had cough (0.3% grade 3-4), 29% had decreased appetite (1.7% grade 3-4), 23% had constipation (0.7% grade 3-4), 11% had pruritus (none grade 3-4). The most common laboratory abnormalities were hyponatremia in 35% (6% grade 3-4) of patients, increased alkaline phosphatase in 27% of patients (2.8% grade 3-4), increased alanine aminotransferase in 23% (2.4% grades 3-4), increased creatinine in 18% (0% grade 3-4), increased thyroid-stimulating hormone in 17%.

Following FDA approval of Nivolumab, reported serious adverse events also include increase risk of transplant-related complications including rejection of solid organ or tissue transplants and onset of graft versus host disease with unknown frequency.

Detailed monitoring for these adverse effects are outlined in section 8, below. While patients are at home, they will be instructed to contact us for any suspected adverse events, whether suspected to be related to the medication or not for further guidance and treatment. Generally, for any reported new symptom or adverse event, non-immune events (such as disease progression) will be ruled out. Once these are ruled out, and the adverse event is deemed to be related to Nivolumab or BMS-986016, the side effects will be generally managed with administration of high-dose (2–4 mg/kg prednisolone equivalent) corticosteroids followed by a tapered dose and interruption of nivolumab therapy. Mild adverse events will be treated with a short course of steroids, followed by interruption of their Nivolumab dose. For non-serious (Grade 1-2) events, that are successfully treated, the patients will be considered for re-entry into the Nivolumab treatment portion of the trial. For Grade 3 events, treatment will generally be continued until the patient returns to baseline, and the patient's steroids will then be tapered to off over 4-6 weeks. A detailed treatment algorithm for potential immune-related side effects is present in appendics B and C; Investigator Brochures. A list of adverse events which will prompt automatic discontinuation of Nivolumab and BMS-986016 are noted in section 8.6, below. Table 3 is provided as a reference for the adverse events from Nivolumab monotherapy in glioblastoma patients, even though the combination treatment for ipilimumab is also reported.

Category Preferred Term	Nivo1 + IPI3 N = 10 n (%)		Nivo3 N = 10 n (%)		Nivo3 + IPI1 N = 20 n (%)	
	Any grade	Grade 3-4	Any Grade	Grade 3-4	Any Grade	Grade 3-4
AEs	10 (100.0)	7 (70.0)	10 (100.0)	4 (40.0)	20 (100.0)	7 (35.0)
Drug-related AEs	10 (100.0)	9 (90.0)	9 (90.0)	0	20 (100.0)	5 (25.0)
Drug-related AEs in > 2 Subjects						
Fatigue	8 (80.0)	1 (10.0)	3 (30.0)	0	11 (55.0)	3 (15.0)
Diarrhea	7 (70.0)	2 (20.0)	1 (10.0)	0	6 (30.0)	1 (5.0)
AST Increased	5 (50.0)	1 (10.0)	0	0	3 (15.0)	2 (10.0)
Lipase Increased	5 (50.0)	5 (50.0)	2 (20.0)	0	1 (5.0)	0
ALT Increased	4 (40.0)	2 (20.0)	0	0	4 (20.0)	2 (10.0)
Vomiting	4 (40.0)	0	1 (10.0)	0	3 (15.0)	0
Amylase increased	3 (30.0)	1 (10.0)	1 (10.0)	0	1 (5.0)	0
Headache	3 (30.0)	0	2 (20.0)	0	4 (20.0)	0
Hyperthyroidism	3 (30.0)	1 (10.0)	1 (10.0)	0	1 (5.0)	0
Nausea	3 (30.0)	0	3 (30.0)	0	3 (15.0)	0
Rash maculo-papular	3 (30.0)	0	0	0	0	0
Rash	1 (10.0)	0	2 (20.0)	0	5 (25.0)	0
Dizziness	0	0	1 (10.0)	0	3 (15.0)	1 (5.0)
Pruritus	0	0	2 (20.0)	0	4 (20.0)	0
SAEs	8 (80.0)	6 (60.0)	6 (60.0)	3 (30.0)	14 (70.0)	7 (35.0)
Drug-related SAEs	7 (70.0)	7 (70.0)	2 (20.0)	0	5 (25.0)	2 (10.0)
Drug-related SAEs in > 1 Subject						
ALT Increased	2 (20.0)	2 (20.0)	0	0	1 (5.0)	1 (5.0)
Colitis	2 (20.0)	2 (20.0)	0	0	1 (5.0)	1 (5.0)
Diarrhea	2 (20.0)	2 (20.0)	0	0	1 (5.0)	1 (5.0)
Hypothyroidism	2 (20.0)	1 (10.0)	0	0	0	0
AEs Leading to Discontinuation	7 (70.0)	5 (50.0)	1 (10.0)	1 (10.0)	1 (5.0)	1 (5.0)
AEs Leading to Discontinuation in > 1 Subject						
Diarrhea	2 (20.0)	1 (10.0)	0	0	1 (5.0)	1 (5.0)
Drug-related Select AEs						
Endocrine AEs	4 (40.0)	2 (20.0)	2 (20.0)	0	3 (15.0)	0
Endocrine AEs in > 1 Subject						
Hyperthyroidism	3 (30.0)	1 (10.0)	1 (10.0)	0	1 (5.0)	0
Hypothyroidism	2 (20.0)	1 (10.0)	2 (20.0)	0	1 (5.0)	0
Gastrointestinal AEs	7 (70.0)	3 (30.0)	1 (10.0)	0	6 (30.0)	1 (5.0)
Gastrointestinal AEs in > 1 subject						

Table 3: Summary of Safety of Nivolumab Alone and in Combination with Ipilimumab in subjects with Recurrent Glioblastoma

Category Preferred Term	Nivo1 + IPI3 N = 10 n (%) Any grade	 Grade 3-4	Nivo3 N = 10 n (%) Any Grade	 Grade 3-4	Nivo3 + IPI1 N = 20 n (%) Any Grade	 Grade 3-4
Diarrhea	7 (70.0)	2 (20.0)	1 (10.0)	0	6 (30.0)	1 (5.0)
Colitis	2 (20.0)	2 (20.0)	0	0	1 (5.0)	1 (5.0)
Hepatic AEs	6 (60.0)	2 (20.0)	0	0	4 (20.0)	2 (10.0)
Hepatic AEs in > 1 Subject						
AST Increased	5 (50.0)	1 (10.0)	0	0	3 (15.0)	2 (10.0)
ALT Increased	4 (40.0)	2 (20.0)	0	0	4 (20.0)	2 (10.0)
Pulmonary AEs	1 (10.0)	0	1 (10.0)	0	1 (5.0)	0
Pneumonitis	1 (10.0)	0	1 (10.0)	0	1 (5.0)	0
Renal AEs	1 (10.0)	1 (10.0)	0	0	0	0
Renal Failure Acute	1 (10.0)	1 (10.0)	0	0	0	0
Skin AEs	5 (50.0)	0	4 (40.0)	0	7 (35.0)	0
Skin AEs in > 1 Subject						
Rash Maculo-papular	3 (30.0)	0	0	0	0	0
Rash	1 (10.0)	0	2 (20.0)	0	5 (25.0)	0
Pruritus	0	0	2 (20.0)	0	4 (20.0)	0
Deaths	8 (80.0)		6 (60.0)		11 (55.0)	
Cause of death						
Disease	6 (60.0)		6 (60.0)		8 (40.0)	
Unknown	2 (20.0)		0		1 (5.0)	
Other	0		0		2 (10.0)	

Source: Preliminary data for CA209143, database lock date, 20-Mar-2015

Note: Safety events were reported between the first dose and 100 days after the last dose of study drug.

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; IPI = ipilimumab; IPI1 = ipilimumab 1 mg/kg; IPI3 = ipilimumab 3 mg/kg; Nivo = nivolumab; Nivo1 = nivolumab 1 mg/kg; Nivo3 = nivolumab 3 mg/kg

Table 3 (Continued): Summary of Safety of Nivolumab Alone and in Combination with Ipilimumab in subjects with Recurrent Glioblastoma

7.17 Summary of risks of BMS-986016 monotherapy and combination of BMS-986016 and nivolumab

7.17.1 Summary of Safety

This section summarizes the potential risks of treatment with relatlimab alone and in combination with nivolumab, and the specific tests, observations, and precautions that are required in clinical studies. As of the clinical cutoff date of 15-Jul-2020, across 3 safety studies (Studies CA224020, CA224022, and CA224034), relatlimab monotherapy has been administered to 66 subjects across multiple doses: 20 mg (8 subjects), 80 mg (13 subjects), 240 mg (27 subjects) and 800 mg (18 subjects) relatlimab, flat dose, Q2W. The safety profile of relatlimab monotherapy appears manageable with no MTD reached. The MAD was 800 mg Q2W. There were 4 drug related SAEs in monotherapy: Grade 3 pneumonitis, Grade 2 pneumonitis, and Grade 3 allergic reaction in monotherapy in Study CA224020 at a dose of 800 mg relatlimab Q2W; and a Grade 3 aseptic meningitis in monotherapy in Study CA224022 at a dose of 800 mg relatlimab Q2W. There was no

apparent relationship in the incidence, severity, or causality of AEs to relatlimab at these dose levels. All AEs were reversible or manageable (in the setting of immune-mediated endocrine events) by withholding drug administration and following treatment algorithms specified in the protocols where applicable. There were 6 Grade 1 to Grade 2 infusion-related reactions with relatlimab monotherapy (1 in Study CA224020 and 5 in Study CA224022), which were manageable and reversible with recommended treatment guidelines in the protocol. A total of 29 subjects died due to malignant neoplasm progression following relatlimab monotherapy (19 subjects in Study CA224020, 9 subjects in Study CA224022, and 1 in Study CA224034). There were 4 drug-related SAEs in monotherapy: Grade 3 pneumonitis, Grade 2 pneumonitis, and Grade 3 allergic reaction in monotherapy in Study CA224020 at a dose of 800 mg relatlimab Q2W; and Grade 3 aseptic meningitis in monotherapy in Study CA224022 at a dose of 800 mg relatlimab Q2W. There was no apparent relationship in the incidence, severity, or causality of AEs to relatlimab at these dose levels. A total of 29 subjects died due to malignant neoplasm progression following relatlimab monotherapy (19 in Study CA224020, 9 in Study CA224022, and 1 in Study CA224034). As of the clinical cutoff date of 15-Jul-2020, treatment with relatlimab in combination with nivolumab has been administered to 1431 subjects in Studies CA224020 (1332 subjects), CA224022 (68 subjects), and CA224034 (31 subjects). The safety profile of relatlimab combined with nivolumab is manageable with currently no MTD reached, as combination dose evaluation remains ongoing.

Across Studies CA224020, CA224022, and CA224034 for both monotherapy and combination therapy, drug-related AEs were reported in 1036 subjects. The most frequent drug-related AEs included fatigue, decreased appetite, pruritus, diarrhea, rash, rash maculo-papular, anemia, asthenia, hypothyroidism, and hyperthyroidism. The types of drug-related AEs, as well as the rates of drug-related AEs, appeared comparable to historical nivolumab monotherapy rates. All drug-related AEs, except for 1 Grade 4 myocarditis (240 mg relatlimab/240 mg nivolumab Q2W), as well as 1 Grade 4 potential drug-induced liver injury, 1 Grade 5 dyspnea, and 1 Grade 3 pneumonitis (all at a dose level of 80 mg relatlimab/240 mg nivolumab Q2W), were reversible or manageable by withholding study drug administration, providing standard medical care, and/or following immune-related AE algorithms.

A list of all SARs reported for relatlimab monotherapy and relatlimab + nivolumab combination therapy is present in Investigator Brochure Appendix 1.

7.17.1.2 Potential Risk Associated with Relatlimab Monotherapy or in Combination with Nivolumab

7.17.1.2.1 Immune-mediated Adverse Events

Monotherapy with relatlimab is investigational and the type and grade of potential effects of inflammatory cells on specific tissues induced by the drug are unknown. The inflammatory effects could include well-characterized events documented for other T cell-directed antibodies including pneumonitis, colitis, hepatitis, nephritis, endocrinopathy, and neurologic and skin AEs. Unanticipated events may also occur. In combination with nivolumab, the frequency, severity, and reversibility of immune-mediated adverse events (IMAEs) appears comparable to those of nivolumab

monotherapy. Recommended IMAE management algorithms are included in the nivolumab (BMS-936558, anti-PD-1 antibody) IB and every relatlimab clinical study protocol. Nivolumab management guidelines should be reviewed. The guidance applies to all IO agents and regimens. A general principle is that differential diagnoses should be diligently evaluated according to standard medical practice. Non-inflammatory etiologies should be considered and appropriately treated. Corticosteroids are a primary therapy for IO drug-related AEs. 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. In addition, refer to any specific management guidance included in the study drug protocol.

7.17.1.2.2 Neurotoxicity

Given the toxicology studies showing choroid plexus inflammation in the setting of combination therapy, diligence regarding clinical monitoring of all subjects for signs and symptoms of neurologic toxicities was emphasized and should be maintained. In the early-phase studies, subjects with active neurological disease, as well as those with a confirmed history of encephalitis, meningitis, or uncontrolled seizures in the year prior to informed consent, have been excluded. As of the clinical data cutoff date of 15-Jul-2020, the observed drug-related AEs with potential for neurotoxic etiology have occurred at rates and with severity comparable to what would be expected with nivolumab monotherapy. The current relatlimab safety experience includes 10 (1 with monotherapy and 9 in combination with nivolumab) cases of aseptic meningitis (which is a rare IMAE with nivolumab monotherapy), neurotoxicity remains a specific IMAE of interest when combining relatlimab and nivolumab. The aseptic meningitis cases all presented with new or worsening headache with diagnosis made based upon increased immune cells found on CSF analysis in the absence of identifiable infectious etiology. Most cases responded to steroid therapy, 1 case responded without steroid therapy, and 1 case recurred during the steroid taper (responded to repeated high dose steroid treatment with taper). Two cases of encephalitis, Grade 3, have occurred with relatlimab in combination with nivolummab. No cases of myasthenia, demyelination or Guillain-Barre Syndrome have occurred. Refer to the specific treatment protocol for individual study-specific eligibility criteria; required procedures and instructions regarding neurotoxicity evaluation and monitoring as guidance may evolve with the increasing clinical and safety experience with combination of relatlimab and nivolumab.

7.17.1.2.3 Potential Infusion Reaction and Cytokine Release

Clinical studies of novel therapeutics have highlighted the risk of cytokine release as a consequence of treatment with some immune-modulatory mAbs. Nonclinical data, however, have demonstrated that relatlimab monotherapy or combination therapy with nivolumab does not induce cytokine release when presented to human PBMCs, regardless of concentration, donor, or incubation time. Consistent with the lack of cytokine release, there was no evidence that relatlimab induced T, B, or NK-cell activation, as measured by surface expression of CD25 and CD69. Thus, the risk of cytokine release after relatlimab administration as a monotherapy or as a combination therapy with nivolumab was assessed to be low based on these data and the minimal effector function mediated

by IgG4 (cross-linking through Fc receptors). Based on preliminary data as of 15-Jul-2020, infusion reactions of low grade (Grade 1 to 2) have been reported in 72 subjects treated across all 3 studies, 5 subjects with relatlimab monotherapy and 67 subjects with combination therapy. Infusion reactions were manageable and reversible following recommended treatment guidelines in the protocol. No cytokine release syndrome has been reported with doses up to 800 mg relatlimab monotherapy Q2W or with combination therapy up to 480 mg relatlimab/480 mg nivolumab Q4W. Nevertheless, relatlimab does have factors/attributes influencing risk, as described in the European Medicines Agency Committee for Medicinal Products for Human Use Guideline on strategies to identify and mitigate risks for FIH clinical studies, with investigational medicinal products (10-Jul-2007). Therefore, in addition to conventional safety assessments, the FIH study monitoring plan included the following precautions:

1) Monitor for infusion reactions during and after administration of relatlimab.
2) Monitor for any potential signs of cytokine release syndrome after the administration of relatlimab.
3) Use a staggered dosing (sentinel subject) approach in the first dose cohorts evaluated in dose escalation to allow for thorough safety evaluation of the first dose prior to administration to subsequent subjects.

Refer to the specific treatment protocol for individual study-specific guidance regarding infusion reactions.

7.17.1.2.4 Opportunistic Infections Due to Immunosuppression

Some subjects may require prolonged treatment with high-dose corticosteroids or alternative immunosuppressants for the treatment of immune-related AEs induced by relatlimab monotherapy or in combination with nivolumab. Subjects may develop opportunistic infections after receiving prolonged treatment with immunosuppressant agents, and these cases may require additional laboratory tests and invasive procedures for proper management. Prophylactic antibiotics and antifungal agents may also be indicated when prolonged treatment with corticosteroids has been planned.

7.17.1.3 Cardiovascular Events

Myocarditis has been observed with nivolumab monotherapy treatment (see nivolumab IB version 19, Appendix 1). No cases of myocarditis have been reported with monotherapy relatlimab treatment. However, 15 cases (1.1% of all grade myocarditis, autoimmune myocarditis, or immune mediated myocarditis have been reported for relatlimab in combination with nivolumab. These include 5 Grade 1, 3 Grade 2, 5 Grade 3, and 2 Grade 4 cases.

Given the clinical significance of myocarditis and the known nonclinical mouse double LAG-3/PD-1 knockout myocarditis phenotype, increased cardiac surveillance during the first 2 months of therapy with troponin measurements (at baseline and prior to study drug administration) was instituted, as well as excluding subjects with a history of myocarditis. Thus, all subjects treated in the expansion cohorts in Studies CA224020, CA224022, and CA224034 have had troponin surveillance as well as ECG monitoring. In this setting, there have been 12 cases of symptomatic myocarditis (5 Grade 2, 5 Grade 3, and 2 Grade 4) and 5 reported cases of asymptomatic troponin increases without evidence of cardiac

dysfunction but with imaging (cardiac-specific MRI or PET) abnormalities consistent with myocardial inflammation (Grade 1 myocarditis per Common Terminology Criteria for Adverse Events, version 4). In all of the symptomatic cases, there was ejection fraction improvement with immunosuppression and discontinuation of study therapy. In the Grade 1 myocarditis cases, study drug was held and precautionary steroid treatment was given without any of the subjects developing evidence of cardiac dysfunction. Subjects experiencing myocarditis may be considered for re-challenge with study medication pending follow-up cardiac evaluations and assessment of cancer disease status.
Refer to the specific study protocol for individual study specific eligibility criteria, required procedures, and instructions regarding cardiac toxicity monitoring and evaluation, as guidance may evolve with the increasing clinical and safety experience with the combination of relatlimab and nivolumab. All troponin elevations or suspected cardiac toxicity should result in immediate repeat testing and cardiac evaluation.
Please note that myocarditis AE management algorithm applicable to studies under version CTCAE v4.0, and relatlimab myocarditis algorithm applicable to studies under CTCTAE v5.0 are included in Appendices B and C; Investigator Brochures for Nivolumab (BMS-936558) and Relatlimab (BMS-986016).

7.17.1.4 Other

Risks from the PK, PD, and clinical safety phlebotomy procedures are standard and include discomfort at the phlebotomy site, bleeding, bruising, and, rarely, infection and fainting. Risks from tumor biopsy include removal of tissue that can elicit pain, pressure, or discomfort in the area where the tissue was taken. Pain and discomfort can last for several hours and up to several days after the biopsy procedure. Subjects may experience bleeding, redness, swelling, bruising, and infection in the area where the tissue was taken or may feel faint or dizzy.

7.18 Risks of Surgery for Tumor Resection—Standard Care

Standard of care neurosurgical techniques will be used for removing the tumor and risks of surgery are standard for intracranial surgery, including aphasia, hemiparesis, and sensory loss. The risk of neurological deficit depends on the proximity of the tumor to language, motor, and somatosensory cortex.

8.0 Subject Safety Monitoring

8.1 Parameters to be monitored

There will be ongoing extensive patient education before and after surgery to ensure that the patient reports all adverse events at the time they occur. All personnel involved in this study are provided with training and written guidelines concerning the definitions of adverse events (AE) and serious adverse events (SAE) and their responsibilities if they believe they have identified an AE/SAE, including reporting any SAE within 24 hours and the completion of the AE/SAE Form.

8.1.1 Study Monitoring Phase 1 – Before surgical resection

Once the patient has been enrolled into the trial, they will be considered to be in Phase 1 of the trial which precedes the surgical resection of the tumor. This period will include the research surgical procedure, the monitoring period in the intensive care unit and inpatient unit and the first dose of Nivolumab. All patients will be monitored for adverse events graded as per the National Cancer Institute Common Terminology Criteria for Adverse Events.

Of note, there is a possibility, though remote, that a frozen section could indicate recurrent tumor, but that the final pathology may only demonstrate treatment effects with no viable tumor. If that is the case, the patient's microdialysis catheters and lumbar drain can be removed in the inpatient unit without returning to the operating room. The patient would then be withdrawn from the study as per 8.6, below, and would be replaced. Microdialysis catheter removal in the inpatient unit has been performed in the setting of research studies quite commonly. The microdialysis catheters in a recently completed study of regadenoson as a blood-brain barrier opening agent in glioblastoma at the NIH were removed in the intensive care unit with no morbidity.

Additionally, as noted in 8.6, below, a patient who experiences a grade 3 or 4 toxicity during Phase 1 of the trial which precludes the completion of the inpatient monitoring period will be withdrawn from the trial. At that time, the patient's microdialysis catheters and lumbar drain would be removed in the inpatient unit. Surgical resection would proceed as per standard of care if still clinically indicated. Otherwise, further standard of care treatment will be offered. The patient can also return to the care of their physicians outside of the NIH.

In addition to our standard post-operative care for our patients, we will be specifically assessing our patients for the following adverse events that are likely to be related to the research surgical procedure – intracerebral hemorrhage, meningitis and wound infection. Any grade of adverse events, as defined by CTCAE version 5.0, which occur within the first 3 patients will trigger an evaluation by the Safety Monitoring Committee to approve the enrollment of the next patient. Any adverse event Grade 2 or higher or any delay in planned surgery in any patient at any point during the pilot trial due to an adverse event related to the experimental procedures in Phase 1 will also trigger an evaluation by the Safety Monitoring Committee (for patients enrolled at NIH) and the Data and Safety Monitoring Board (for patients enrolled at the Emory Site) to approve the enrollment of the next patient.

Infusion reaction: Monitoring for an infusion reaction will begin on day 3 of the protocol within the patient's inpatient stay. This monitoring will consist of clinical observation, with q15 minute vital signs, during the infusion and for the first two hours after infusion. Specifically, we will be checking the patients for fever, chills, shaking, itching, rash, hypertension or hypotension or difficulty breathing during the administration of Nivolumab or immediately afterwards (within the subsequent 2 hours).

For infusion reactions, the patients will be treated according to I-O AE Algorithms from Appendices B and C; Investigator Brochures for Nivolumab (BMS-936558) and Relatlimab (BMS-986016). Generally, Grade 1 infusion reactions will be observed. Grade 2 or 3 infusion reactions will prompt interruption of the patient's infusion until the observed symptom returns to the level of a Grade 1 reaction. Patients will be given 25mg of Benadryl IV and 650 mg of Tylenol PO for any grade 2 or 3 infusion reaction. The Benadryl can be repeated once for symptom resolution until the reaction returns to a level of Grade 1. If a patient has a Grade 2 or 3 reaction, subsequent infusions will be preceded by 25mg of Benadryl IV and 650mg of Tylenol PO 30 minutes prior to dosing. Grade 4 reactions will cause discontinuation of the therapy.

Additionally, the following safety monitoring procedures will be undertaken during Phase 1 of the trial:

-The patient will undergo an immediate post-operative neurological examination within 4 hours of the completion of the case to rule out any neurological morbidity after the research biopsy and microdialysis catheter placement;
-The patient's dressing will be examined on a daily basis for signs of infection including increased tenderness, foul odor, discharge or discoloration of the dressing.
-A CT scan will be performed in the immediate post-operative period within 8 hours of the completion of the case to ensure lack of hemorrhage at site of catheter implantation;
-In the intensive care unit, the patient will undergo monitoring in the ICU for 24 hours after microdialysis catheterization, and then an inpatient stay with a one-on-one sitter (7 days) after biopsy
-The patient will have q2hr neurological checks for the first 24 hours after surgery
-During the remainder of the patient's time while admitted, they will have q4hr neurological checks while awake;
-If any significant neurological changes are found on the neurological checks, the patient will be sent for a STAT head CT to rule out late onset of hemorrhage;
-Patient will have q4hr temperature checks while admitted
-Patient will be watched for signs and symptoms of adrenal insufficiency while admitted during nursing checks and daily physical exams
-Patients will have skin examinations and will be asked for signs and symptoms of immune-mediated rash while admitted
-CSF will be collected from the lumbar drain for safety monitoring on Mondays and Thursdays while the patient is in the inpatient unit, as long as the lumbar drain remains in place. We will send a cell count, protein, glucose, gram stain and culture from each sample
-Patients will undergo a CBC with differential daily while in the inpatient unit to watch for signs of infection.
-Patients will be monitored with metabolic panels daily during the microdialysis monitoring period
-Patients will undergo liver function testing on day 3 prior to study drug administration.
-Patients will undergo CPK testing on day 3 prior to study drug administration.
Lab values that would preclude dosing of Nivolumab include:
-Creatinine > Upper limit of normal and > 1.5x baseline

-AST > 3.0 times upper limit of normal
-ALT > 3.0 times upper limit of normal
-Total bilirubin > 1.5 times upper limit of normal
-WBC > 2.0 times upper limit of normal
-CPK > 2.0 times upper limit of normal
-Patients will be followed with QAC and QHS accuchecks for glycemic control while admitted
-If the flow through either of a patient's microdialysis catheters stops, this catheter will be removed in the inpatient unit.

8.1.2 Study Monitoring Phase 2-Surgical Resection and follow-up period:

Once the patient goes into surgery for tumor resection, they will be considered to be in Phase 2 of the trial. The patient's post-resection care until Day 17 (+/- 2 days) will be carried out largely in accordance with standard of care. The patient will stay in the ICU for at least 24 hours after the surgical resection. The patient will receive a post-operative MRI to ensure adequate resection and lack of surgical complications that would be evident on MRI. Once the patient is deemed stable to transfer from the ICU, they will then be monitored in the NPCU for 2-5 days, on average, before being discharged to home.

While being monitored as an inpatient, patients will be specifically evaluated by the medical and nursing staff for symptoms and signs of 1) focal neurologic dysfunction, such as weakness, sensory loss, and aphasia; 2) general neurologic dysfunction, such as somnolence; 3) increased intracranial pressure; and 4) infection of the wound, brain, or meninges.

During the study follow-up period, from discharge from the hospital through the discontinuation of study medications, monitoring will be carried out in accordance with the prescriber information for Nivolumab. Additional precautions will be taken due to the addition of BMS-986016 to the dosing regimen.

Pre-infusion monitoring will include a rigorous evaluation prior to each dose of study medications. Prior to the first dose, patients will undergo an echocardiogram, free T3 and T4 and baseline ACTH and AM cortisol. Prior to each dose, lab tests will include:
-complete blood count with differential
-liver function tests with AST, ALT and Tbili,
-metabolic panel with creatinine,
-CRP
-TSH
-CPK (prior to the first dose of nivolumab)
-troponin (prior to the first five doses of BMS-986016),
-EKG (prior to the first five doses of BMS 986016)

Prior to each dose, patients will also undergo:

-History and physical examination with detailed neurological examination, skin examination and evaluation for signs of adrenal insufficiency and evaluation for respiratory compromise;
-Vital signs
-MRI (at every other treatment visit, starting on Day 17 (+/- 2 days));

Lab values that would preclude dosing of Nivolumab and BMS-986016 include:
-Creatinine > Upper limit of normal and > 1.5x baseline
-AST > 3.0 times upper limit of normal
-ALT > 3.0 times upper limit of normal
-Total bilirubin > 1.5 times upper limit of normal
-WBC > 2.0 times upper limit of normal
-CPK > 2.0 times upper limit of normal

Prolonged QT interval, arrhythmia, T-wave abnormalities or any other concerning ECG findings will prompt a consultation to cardiology to rule out pre-existing or newly developed cardiac pathology prior to dosing of the study drugs.

Monitoring for an infusion reaction and cytokine release on Day 17 (+/- 2 days) of the protocol will consist of clinical observation in the day hospital ward or 7SW (if the patient remains admitted to the hospital) of the NIH Clinical Center. The total infusion monitoring time will be expected to last 4-5 hours for most patients. First, the patient will receive Nivolumab as an IV infusion over 30 minutes. Patients with a prior Grade 2 or 3 infusion reaction will have pretreatment with 25mg of Benadryl IV together with 650mg of Tylenol PO. During both infusions, patients will have q15 minute vital signs, including temperature, pulse, blood pressure and pulse oximetry. BMS-986016 infusion will begin 15-30 minutes after completion of the Nivolumab infusion. BMS-986016 will then be given as an IV infusion over 60 minutes with ongoing monitoring. The patients will also be monitored for two hours after completion of the BMS-986016 in the day hospital or 7SW. Specifically, we will be checking the patients for fever, chills, shakes, itching, rash, hypertension or hypotension or difficulty breathing during the administration or immediately afterwards (within the following 2 hours). The patients will be treated according to I-O AE Algorithms from Appendices B and C; Investigator Brochures for Nivolumab (BMS-936558) and Relatlimab (BMS-986016). Generally, Grade 1 infusion reactions will be observed. Grade 2 or 3 infusion reactions will prompt interruption of the patient's infusion until the observed symptom returns to the level of a Grade 1 reaction. Patients will be given 25mg of Benadryl IV and 650 mg of Tylenol PO for any grade 2 or 3 reaction. The Benadryl can be repeated once for symptom resolution until the reaction returns to a level of Grade 1. If a patient has a Grade 2 or 3 reaction, subsequent infusions will be preceded by 25mg of Benadryl IV and 650mg of Tylenol PO 30 minutes prior to dosing. Grade 4 reactions will cause discontinuation of the therapy. Patients will be instructed to inform the clinical team of any symptoms including, but not limited to, fever, chills, itching, shortness of breath, stomach discomfort, diarrhea, new neurological symptoms, seizures, changes in urinary function, chest pain, palpitations, rashes or any other significant concern in between drug doses.

Additionally, we will follow the I-O AE Algorithms from Appendices B and C; Investigator Brochures for Nivolumab (BMS-936558) and Relatlimab (BMS-986016). Additionally lab tests will be ordered as clinically indicated.
Adverse effects will be monitored systematically throughout the study with formal assessments at each evaluation visit. In addition, subjects will be encouraged to contact the investigators by telephone any time there is any concern about side effects. If necessary, an unscheduled clinical visit with the investigator will be arranged. Patients will have access to clinicians day and night for the duration of the study. The Principal Investigator, in collaboration with the Safety Monitoring Committee (for patients enrolled at NIH) and the Data and Safety Monitoring Board (for patients enrolled at the Emory Site), will be responsible for monitoring data collected to ensure the safety of subjects.

Monitoring for safety of individual subjects during participation in study procedures will be performed by the medical staff and the research nurses.

8.2 Toxicity Criteria

Toxicities and adverse events will be recorded and categorized according to severity, relationship to procedure, and relationship to the study procedure or study drugs. Level of toxicity is based on the NCI CTCAE criteria.

8.3 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, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for participant). The patient will continue to be followed until the outcome of the pregnancy can be determined, and will then continue with routine surveillance imaging as per the protocol.

8.4 Overdose

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

8.5 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 nonserious or serious AE, as appropriate, and reported accordingly.

8.6 Criteria for Individual Subject Withdrawal from the Study

Subjects may withdraw from the study at any time for the following reasons:

1. Subject's prerogative: The subject desires to discontinue participation in this study.

2. Investigator's prerogative: The subject is unwilling or unable to comply with the protocol.

Subjects must discontinue study drugs for the following reasons:

1. The biopsy indicates that the patient does not have a true pathological recurrence of their tumor
2. The patient experiences a grade 3 or 4 toxicity during Phase 1 of the trial which precludes the completion of the inpatient monitoring period
3. The patient experiences a toxicity consistent with the Nivolumab prescribing information that requires discontinuation of Nivolumab. Such toxicities will be deemed sufficient to discontinue both Nivolumab and BMS-986016. Specifically, these toxicities are:
    a. Immune-mediated pneumonitis: Grade 3 or 4 immune-mediated pneumonitis will prompt automatic discontinuation of study drugs
    b. Immune-mediated colitis: Grade 4 immune-mediated colitis will prompt automatic discontinuation of study drugs
    c. Immune-mediated hepatitis: Grade 3 or 4 immune-mediated hepatitis will prompt automatic discontinuation of study drugs
    d. Immune-mediated endocrinopathies: Grade 4 immune-mediated hypophysitis; Grade 3 or 4 immune-mediated adrenal insufficiency; and Grade 4 hyperglycemia will prompt automatic discontinuation of study drugs. Additionally, thyroid function will be followed, and thyroid hormone replacement will be given as needed
    e. Immune-mediated nephritis and renal dysfunction: Grade 4 serum creatinine elevation will prompt automatic discontinuation of study drugs
    f. Immune-mediated skin adverse reactions: Grade 4 rash will prompt automatic discontinuation of study drugs
    g. Immune-mediated encephalitis: Grade 4 encephalitis will prompt automatic discontinuation of study drugs
    h. Infusion reactions: Grade 3 or 4 infusion reactions will prompt automatic discontinuation of study drugs
    i. In addition to the above, due to the report of myocarditis in one patient on the combination of BMS-986016 and Nivolumab, Grade 4 myocarditis will prompt automatic discontinuation of study drugs
4. Clinical progression
5. Confirmed radiological progression and investigator determination that the patient is no longer benefiting from treatment with the study treatment

If study drug is discontinued, the patient will enter the survival follow-up phase of the study.

Additionally, if a patient is removed from the study for clinical or radiological progression of disease, we will perform a follow-up assessment at the next scheduled imaging visit and no less than 4 weeks after the prior assessment of disease progression.

9.0 Outcome Measures

9.1 Primary outcome measure

The primary outcome measures are:

1. The proportion of patients that have a rise in interferon gamma levels within the tumor microenvironment of 4 pg/ml or higher before the first dose of Nivolumab as compared to the 5th day after treatment with Nivolumab.
2. The safety of the use of microdialysis catheters for immune monitoring in recurrent glioblastoma patients
3. The safety of the combination of nivolumab and BMS-986016 in recurrent glioblastoma patients

9.2 Exploratory outcome measures

Exploratory outcome measures and include:

1. The change in interferon gamma expression from before, during and after Nivolumab treatment in the brain microenvironment, cerebral spinal fluid and the blood.
2. The difference between the IHC staining for CD3, CD4, CD8, CD20, CD45RO, CD57, CD68, FOXP3, Granzyme B, LAG3, PD-1, CD14, CD33, CD163, and CD206 before and after Nivolumab therapy. These markers will be evaluated as density of cells, defined as the number of positive cells per mm^2. PD-L1 expression will be evaluated in tumor cells using H-score, which includes the percentage of positive cells showing membrane staining pattern (0–100) multiplied by the intensity of the staining (0 to 3+), with a total score ranging from 0 to 300. The final score for each marker will be expressed as the average score of the areas analyzed within the tumor region (tumor center).
3. Progression free survival and overall survival of recurrent glioblastoma patients after treatment. Progression free survival is defined as the time length from the first dose of study drug with recurrence to progression as defined by iRANO criteria, and the overall survival is defined as the time length from the first dose of study drug to death. If a lesion is 25% larger but there remains a question as to disease progression or pseudoprogression due to treatment, the patient can remain on treatment until this can be defined. If true progression occurred earlier, the earlier date will be used for censorship. If patient is lost to follow-up prior to documented progression or death, patient will be censored as of the date of the last adequate evaluation (MRI and exam). If patient dies prior to documentation of progression, progression will be defined as the date of death, if patient has been attending regularly scheduled follow-ups.
4. The difference in progression free survival and overall survival between patients who have an interferon gamma response versus those that do not have one.

5. The difference in the immune cells and secreted factors of the tumor environment as compared to the immune cells and secreted factors of the cerebral spinal fluid, blood and potential bone marrow in response to this treatment

10.0 Statistical Analysis

10.1 Data analysis

Primary goals

1) To determine the proportion of patients who have an increase of interferon gamma production within the tumor microenvironment from before and after therapy with the immune checkpoint inhibitor, nivolumab.
The change will be dichotomized as increase vs. no increase with the threshold of 4pg/ml. The proportion of the patients with increase will be evaluated by exact binomial test.

2) To evaluate the safety of using brain tumor microdialysis to monitor response to immune modulators in patients with recurrent glioblastoma.
Descriptive statistics, such as frequency and percentage, will be used to evaluate the safety of these procedures.

3) To evaluate the safety of the combination of Nivolumab and BMS-986-016 in the treatment of recurrent glioblastoma patients.
Descriptive statistics, such as frequency and percentage, will be used to evaluate the safety of these treatments.

Exploratory Goals

1) To determine the change in interferon gamma production within the tumor microenvironment and in the rest of the body from before and after therapy with the immune checkpoint inhibitor, nivolumab.
A one-sample t-test or Wilcoxon signed rank test will be performed to evaluate the change (continuous) over time (between pre- and post-treatment) in the outcome measures

2) To evaluate the pathological response of the immune microenvironment of brain tumor tissue to the first dose of Nivolumab
A one-sample t-test or Wilcoxon signed rank test will be performed to evaluate the change between pre- and post-treatment for each marker evaluated.

3) To evaluate the clinical response (progression free survival, overall survival) of recurrent glioblastoma patients to this treatment
The Kaplan-Meier method will be used to estimate the survival function and generate survival curves. The study is not powered to detect significant differences in survival between the patients in this cohort and historical controls.

4) To describe the difference in survival between responders and non-responders
Descriptive statistics will be used. The study is not powered to detect significant differences in survival between the patients in this cohort and historical controls.

5) To examine the differences in the immune cells and secreted factors of the tumor environment as compared to the immune cells and secreted factors of the cerebral spinal fluid, blood and potential bone marrow in response to this treatment
Repeated measures ANOVA or Friedman test will be performed to evaluate the change (continuous) over time (between pre- and post-treatment) and between brain compartments (or between brain and the peripheral immune system, or between the two different micro dialysis catheters).

10.2 Power analysis

The power analysis was performed using the primary outcome, which is defined as increase vs. no increase based on the difference in expression of interferon gamma between baseline and the 5th day after nivolumab treatment with the threshold of 4pg/ml. The hypothesis is that the proportion of the patients with increase is greater than 0.2 . The expected proportion is 0.7. The following table shows the estimated power based on 10 subjects for different null and observed proportions using the two-sided exact binomial test.

Table 6 - The estimated power of 10 subjects for different null and observed proportions

Null Hypothesis	Expected Proportion	Actual Alpha	Estimated Power
0.1	0.9	0.0128	>.999
	0.8	0.0128	>.999
	0.7	0.0128	0.989
	0.6	0.0128	0.945
0.2	0.9	0.00637	0.998
	0.8	0.00637	0.967
	0.7	0.00637	0.85
0.3	0.9	0.01059	0.987
	0.8	0.01059	0.879
0.4	0.9	0.01834	0.930

Table 6 indicates that a sample size of 10 subjects: (a) will have 85 power to reject the null hypothesis: p<=0.2 when >=7 subjects are found to have increased interferon gamma in five days after the treatment and (b) will have 93.0% power to reject the null hypothesis: p<=0.4 when >=9 subjects are found to have increased interferon gamma.

Table 7 - 95% confidence limits (CL) for proportions with a sample size of 10 subjects.

Observed proportion	95% CL	
1	0.6915	1
0.9	0.555	0.9975
0.8	0.4439	0.9748
0.7	0.3475	0.9333 (a)
0.6	0.2624	0.8784
0.5	0.1871	0.8129
0.4	0.1216	0.7376
0.3	0.0667	0.6525

Table 7 presents the 95% confidence limits for the observed binomial proportion 0.3 to 1.0 based on a sample size of 10 subjects. For a sample size of 10 subjects, if 7 subjects are observed with increased interferon gamma in five days, the interval of (0.35, 0.93) will have 95% probability to include the true proportion (a).

11.0 Human Subjects Protection

11.1 Subject Selection

Enrollment will be equitable among those individuals who meet the inclusion criteria and will not be based on race, ethnicity, or gender.

Patients who are considered for this protocol are those with recurrent glioblastoma who are surgical candidates. The patient population for this study is restricted to patients with recurrent glioblastoma, as experimental therapy is warranted in the care of these patients and surgical resection is indicated for these patients.

11.2 Justification for exclusion of participants less than 18 years old

Participants under the age of 18 years are excluded because glioblastoma is rarely seen in the younger population. An adult's immune system is also different so the effects of immune modulators in children and young adults may also be different than in adults.

11.3 Justification for exclusion of other vulnerable subjects

Subjects who lack consent capacity due to cognitive impairment, or patients with mental disorders or with cognitive impairment that renders them unable to give consent will be excluded because they will be unlikely to be able to safely participate in ongoing microdialysis monitoring in the intensive care unit.

Subjects who do not speak English or Spanish will be excluded from the protocol, as there is a need for close communication between the investigators and subjects, given the nature of the interventions in this trial.

Pregnant and breastfeeding women are excluded as we do not know if Nivolumab can harm a developing fetus or breastfeeding infant.

11.4 Safeguards for vulnerable populations and sensitive procedures

Adults who participate in the protocol are required to sign a durable power of attorney because of the possibility that they may become unable to make decisions about research participation and medical care during the course of participation.

If a patient loses consent capacity after enrollment but before completion of the monitoring period in the inpatient unit and the subsequent tumor resection, they will proceed through the trial, granted that the patient opts to sign assent and their durable power of attorney believes that they would want to proceed. If a patient loses capacity after enrollment but before completion of the monitoring period in the inpatient unit and refuses to sign the assent or the durable power of attorney determines that the patient would not want to continue with the trial, they are removed from the trial and replaced. Those who lose capacity after enrollment and after completion of the monitoring period and subsequent tumor resection will be given the opportunity to continue in the study. In this latter case, a memorandum will be generated that includes a justification for their continued involvement in the protocol, and their DPA will be referred to for further decision-making while at the National Institutes of Health.

The decision process discussed with the DPA will include a summary of risks of the research and likelihood of benefit (if any) for adults unable to consent. The procedure utilized for obtaining the surrogate consent (assent) will be documented, including the procedures for allowing dissent.

The Ability to Consent Assessment Team (ACAT) will be used to provide additional safeguards and protections for vulnerable subjects. If there are any questions about capacity to consent, the Ability to Consent Assessment Team (ACAT) may be consulted. The ACAT may also be consulted, at the discretion of the PI on an ad hoc basis if the subject's mentation changes over the course of the study.

Safeguards for females of childbearing potential: Pregnant women may not be enrolled in this trial. Pregnancy testing will be performed during the initial screening portion of the trial, and every month, prior to MRI scanning, subsequently; and contraception is required during the duration of the trial. Contraception is also required for the 24 weeks after the last exposure to the study drug for those patients of childbearing potential. Subjects who are capable of fathering children will need to use effective contraception until 33 weeks after the last exposure to the study drug.

Safeguards for pediatric patients: Pediatric patients will not be enrolled in this trial.

Safeguards for NIH employees: Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH,

2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff. 4) Independent consent monitoring will be provided by the NIH HSPU. 5) The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures. No compensation will be provided for this protocol.

Sensitive information will be collected from employees participating in the study, including pregnancy status, full medical history, family medical history, psychiatric history, substance abuse history and other components of a full medical history. Surgical treatment will require the employee to be disrobed in front of other clinical center staff. These sensitive procedures will be disclosed to employees at the time of enrollment. Sensitive information that is made part of the medical record will be accessible to the NIH staff that are involved in the medical care of the patient. Whenever possible, only coded data will be made available to those research staff involved in data processing, and protected health information will only be shared as needed to match patient data to other clinical data for processing.

Safeguards for subjects who do not speak English: Subjects who speak Spanish as their primary language will be given the option of reading and signing the Spanish consent for this protocol. A Spanish interpreter will be used to maximize communication between the investigators and participants during the consent process and to help the investigators answer participant questions. An interpreter will be used for all procedures for those subjects who do not understand English fluently. If a Spanish interpreter is not available, Spanish-speaking staff or the interpreter phone line will be used. An interpreter will also be available throughout the duration of the study and at any follow-up appointments for all patients.

Sensitive procedures: No sensitive procedures (deception, placebo, medication withdrawal, etc.) will be used.

12.0 Anticipated Benefit

Subjects will receive Nivolumab and BMS 986016, agents that may have activity for the treatment of brain tumors, though this is not yet proven. This may present the possibility of direct benefit if the therapy slows the progression of the tumor, but any benefit may be short-lived. Increased knowledge of the immune activity of this treatment will not directly medically benefit the patient, but may guide further treatment for future glioblastoma patients.

12.1 Overall risk and benefit consideration

The risks are reasonable in relation to anticipated benefit

13.0 Consent Documents and Process

13.1 Designation of those obtaining consent

Study investigators designated as able to obtain consent on Study Personnel Page, will obtain informed consent. Only advanced medical practitioners that are credentialed at the level of a nurse practitioner, physician's assistant or physician will be permitted to obtain consent. All study investigators obtaining informed consent have completed the NINDS HSPU 'Elements of Successful Informed Consent' training.

13.2 Consent procedures

Cognitive capacity to consent will be determined at the time of enrollment by the treating advanced medical practitioner. At the time of enrollment, a lack of consent capacity due to cognitive impairment is a state that would make them incapable of understanding the explanation of the procedures in this study, and the patient would be excluded from enrollment. If there is any question of the ability of the patient to consent, the ACAT will be consulted. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

Adults must be able to provide consent upon study entry.

The risks and benefits of the proposed study will be explained to the subject. This discussion will include the discussion of the procedures that are research only as opposed to the standard of care. We will also discuss the potential treatments available in the community and other clinical trials available at the National Institutes of Health such as additional radiation, alternative chemotherapeutic agents or surgical treatment without additional therapy. A copy of the informed consent document will be given to the patient. The consent documents contain all required elements and are attached separately. Copies of the consent documents will be maintained in the research records. The original consent will be placed in the patient's medical record. Adults must appoint a durable power of attorney (DPA) upon study entry. If there is any question of the ability of the DPA to consent, the ACAT will be consulted. The DPA will only be invoked if medically necessary due to a new lack of decision-making capacity that occurs during the trial as a result of medical treatment or progression of disease.

It is possible that as a result of the research or standard of care neurosurgical procedures that the patient could lose consent capacity over the course of the study. A patient could also become incapacitated as a result of progression of their lesion during the continued Nivolumab treatment in the follow-up period. If a patient loses consent capacity after enrollment but before completion of the monitoring period in the inpatient unit and the subsequent tumor resection, they will proceed through the trial, granted that the patient opts to sign assent and their durable power of attorney believes that they would want to proceed. If a patient loses capacity after enrollment but before completion of the monitoring period in the inpatient unit and refuses to sign the assent or the durable power

of attorney determines that the patient would not want to continue with the trial, they are removed from the trial and replaced. Those who lose capacity after enrollment and after completion of the monitoring period and subsequent tumor resection will be given the opportunity to continue in the study. In this latter case, a memorandum will be generated that includes a justification for their continued involvement in the protocol, and their DPA will be referred to for further decision-making while at the National Institutes of Health.

We will present any patients who lose consent capacity with an assent form to continue with their participation in the study. The patient's DPA or other appropriate representative would be taken through the consent process at this time. A copy of the assent form is attached. Written assent will be obtained from those without consent capacity. Any dissent from the patient will be respected.

Subjects who speak Spanish as their primary language will be given the option of reading and signing the Spanish consent for this protocol. A Spanish interpreter will be used to maximize communication between the investigators and participants during the consent process and to help the investigators answer participant questions. An interpreter will be used for all procedures for those subjects who do not understand English fluently. If a Spanish interpreter is not available, Spanish-speaking staff or the interpreter phone line will be used. An interpreter will also be available throughout the duration of the study and at any follow-up appointments for all patients.

13.3 Consent documents

The consent form contains all required elements. The following consent documents are submitted with this protocol – subject consent (English and Spanish) and assent (English and Spanish).

14.0 Data and Safety Monitoring

14.1 Data and safety monitor

This protocol will utilize a Safety Monitoring Committee (SMC) (for patients enrolled at NIH) and the Data and Safety Monitoring Board (for patients enrolled at the Emory Site), and an Independent Medical Monitor (IMM) to review AEs and safety reports. The Safety Monitoring Committee (for patients enrolled at NIH) will be composed of:

- Russell Lonser, MD: Chairman of Neurosurgery, OSUMC, Former Chief of Surgical Neurology Branch, NINDS; considerable experience in invasive neurosurgical trials at the NIH Clinical Center and elsewhere including first in human studies, surgical practice focuses on neurosurgical oncology
- Richard Sherry, MD: Staff Clinician, Surgery Branch, National Cancer Institute, National Institutes of Health; extensive experience as a surgeon in the design and conduct of clinical trials of immunotherapies in cancer patients.
- Kilian Salerno, MD: Staff Clinician, Radiation Oncology Branch, National Cancer Institute, National Institutes of Health: experienced clinician in the treatment of primary and recurrent glioblastoma patients with radiation therapy.

- A backup SMC member will be provided by the IND Sponsor, if required. The backup SMC member will have experience with immunotherapy or treatment of primary and recurrent glioblastoma patients.

The DSMB members and Chairperson were appointed by NINDS Clinical Trials Unit (CTU) and approved by the CD and IRB. The members reflect the disciplines and medical specialties necessary to interpret the data from the study and ensure participant safety. The membership of the DSMB are outlined in the DSMB Charter.

Dr. Lauren Reoma, MD, NINDS Staff Clinician, will serve as the IMM for this trial. A backup IMM will be supplied by the IND sponsor, if required. The backup IMM will be an NINDS clinician with knowledge of FDA regulation and immune therapy.

14.2 Data and safety monitoring plan

Study investigators will evaluate the safety of study subjects throughout the conduct of the study and respond to adverse events (AEs) in a timely manner. The IMM will be sent once weekly written patient status reports during the inpatient admission of any study patient for the study procedures (Week 0 through Week 2), or more frequently if needed. The IMM may also be consulted in person and as needed to discuss clinical issues. In person consultations with the IMM will be documented.

The DSMB serves as the Sponsor's medical monitor and will make final determinations of relatedness and expectedness on behalf of the Sponsor (NINDS). The DSMB is charged with reviewing all safety and efficacy data at a twice-yearly meeting. This meeting may take place in person, as a teleconference, or via email. The DSMB may be consulted in person and as needed to discuss clinical issues. The DSMB will operate under the rules of an approved charter that will be reviewed during the first DSMB meeting.

All SAEs, or Grade 3 or higher AEs that are at least possibly related to the research will be forwarded to the DSMB as soon as possible but no later than 7 days after the PI learns of the event, and advice will be sought on whether or not any changes in the research plan are warranted.

The Safety Monitoring Committee (SMC) (for patients enrolled at NIH) and the Data and Safety Monitoring Board (for patients enrolled at the Emory Site) will meet at intervals specified by the needs of the trial, via conference call, email, or web-meeting by a password protected phone line/secured email/web meeting. The SMC will meet at the following intervals:

- Prior to enrolling the first subject to confirm the monitoring plan
- After each subject completes day 24 +/- 2 days
- At least semiannually while the study is open to enrollment or has participants in active follow-up

Additional meetings may be scheduled when necessary for adequate monitoring. Reports containing cumulative safety data and a summary of study progress will be provided to the SMC and DSMB at the semiannual meetings. Participant specific safety reports will be provided to the SMC and DSMB after each subject completes day 24 +/- 2 days. For the first 3 patients, the SMC and DSMB will review the available data at day 24 +/- 2 days prior to approving the dosing of the next subject. After that, the SMC will review the data from day 24 +/- 2 days within 4 weeks after completion of the visit, however further patients may initiate dosing before the SMC and DSMB review. SMC and DSMB meeting minutes will be documented and reported to the Principal Investigator, and to the IRB, through the PI, and NINDS Clinical Director. Data review will be coded, and no interim analysis is planned.
After each meetings, the DSMB chair will provide a report to the PI and NINDS Clinical Trials Unit (CTU) and Office of the Clinical Director (OCD).

All SAEs, or Grade 3 or higher AEs that are at least possibly related to the trial will be forwarded to the SMC (for patients enrolled at NIH) and the DSMB (for patients enrolled at the Emory Site) and the IMM as soon as possible but no later than 7 days after the PI learns of the event, and advice will be sought on whether or not any changes in the research plan are warranted.

This multi-institutional study will be monitored in accordance with the Sponsor's Data Safety Monitoring Charter.

14.3 Criteria for stopping the study or suspending enrollment or procedures

If, in the judgment of the PI, IMM, the SMC or DSMB, a specific study procedure is yielding frequent unexpected or adverse outcomes, that procedure will be suspended until a review can be undertaken in consultation with the IRB. Specifically, for the first 3 patients, if any grade toxicity that is related to the study procedure occurs during Phase 1 of the trial, the Safety Monitoring Committee will be convened to discuss study procedures and to see if any changes need to be made. Any adverse event Grade 2 or higher or any delay in planned surgery due to an adverse event related to the experimental procedures in Phase 1 at any point of the trial will also trigger an evaluation by the Safety Monitoring Committee to approve the enrollment of the next patient. This meeting will need to occur within 2 weeks of the adverse event. Enrollment will be halted until this meeting is convened.

After the first 3 patients, the study will be automatically paused and further enrollment will be halted for any Grade 3 or higher SAE in Phase 1 of the trial. Enrollment will be halted until this meeting is convened and the IRB has reviewed and approved the findings of the committee. Depending on the results of this meeting, the procedure in question may be dropped from the protocol via an amendment, or specific language may be added to the protocol and consent forms to reflect the changing risk level.

15.0 Quality Assurance

15.1 Quality Assurance Monitor

The Principal Investigator will ensure that:

- the protocol is being correctly followed
- changes to the protocol have been approved by the IRB
- accurate, complete, and current records are being maintained and are secure
- subject withdrawal or study failure is noted in the records and
- informed consent has been correctly documented

15.2 Quality Assurance Plan

A Contract Research Organization (CRO) will monitor this protocol. On-site monitoring will be carried out by the CRO. An initial visit will be conducted by the CRO, following final approval of the protocol by the IRB and FDA. During the initial visit, the study team and the monitor will determine the frequency of monitoring visits. The PI/sponsor via the CRO, will be responsible for providing adequate oversight of the investigation to ensure adequate protection of the rights, welfare, and safety of human subjects and the quality and integrity of the resulting data.

This multi-institutional study will be monitored in accordance with the Sponsor's QA Monitoring Plan.

16.0 Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

Reportable events will be tracked and reported in compliance with Policy 801.

16.1 FDA Reporting Criteria

The protocol's IMM will act as IND Sponsor's (NINDS) Medical Monitor.

Additionally, we will follow the reporting requirements as outlined in 21CFR312.32. The sponsor will notify the FDA of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days from initial receipt of such information using the MedWatch Form 3500a.

The Sponsor is also responsible for reporting any of the following to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting using the MedWatch Form 3500a: suspected adverse reaction that is both serious and unexpected; any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings from animal or in vitro testing that suggest a significant risk in humans exposed to the drug; or clinically significant increase in the rate of a serious suspected adverse reaction over that listed in the protocol or either investigator brochure. If FDA requests any additional data or information, the sponsor must submit it to the FDA as soon as possible, but no later than 15 calendars days after receiving the request.

16.2 Reporting of protocol deviations

For safety monitoring, during the microdialysis monitoring period and during subsequent protocol visits, safety checks performed within +/- 5 minutes of stated guidelines will not be considered protocol violations. More than two missed safety checks during infusion monitoring during the microdialysis monitoring period in Phase 1 of the study or during any individual subsequent infusion during Phase 2 of the study will be considered a protocol deviation.

Patients who miss one or more doses of study drug due to treatment of inflammatory side effects will not be censored if these side effects are mild and/or treatable (Grade 1 or 2). Such patients will continue on the study drug when this is safe. This will not be considered a study deviation. Patients who miss one visit will be counseled that further missed follow-up will cause for them to be removed from the Nivolumab and BMS-986016 treatment portion of the trial. Patients that miss two or more consecutive treatments with Nivolumab and BMS-986016 will be censored at the time that they are lost to follow-up. These would be reported as study deviations. We do not expect omissions such as these to affect the primary study outcomes, as these are focusing on the effects of the treatment in the immediate post-surgical period. Based on our experience working with the Neuro-oncology Branch in this patient population, we do not expect to lose many patients in this treatment period.

In the event that circumstances beyond our control preclude the travel of one or more of the study participants to the NIH Clinical Center for a visit, or otherwise would put their health at greater risk (i.e. epidemic or pandemic), we will endeavor to establish capabilities for remote visits. Specifically, for safety visits, the study team will arrange for local laboratory studies to be done, which include comprehensive metabolic panel (with creatinine), GGT, TSH, CPK, CRP, Liver function tests (including Tbili, AST and ALT), CBC with diff, MRI Brain with/without contrast, and conduct a telehealth visit with the patient. This telehealth visit will adhere to HHS guidance (https://www.hhs.gov/hipaa/for-professionals/special-topics/emergency-preparedness/notification-enforcement-discretion-telehealth/index.html) and can use all telehealth modalities under the good faith provision during a pandemic/epidemic as allowed by HHS for care of study participants. Unscheduled visits, due to safety, may also be conducted remotely through telehealth and/or offsite laboratory and radiologic studies and/or in conjunction with a local physician with the supervision of the study PI. The laboratory studies will be performed through LabCorp or with the local physician, and results will be sent to the study team for safety monitoring and oversight. Radiology studies will be arranged through a local, non-study physician, or directly by the study team, at a site close to the participants home area if travel to the NIH is not possible. If a patient is scheduled for an infusion, the study team will engage with a local physician to oversee the infusion and study visit at the outside non-research site under the direction of the study PI. The outside physician would be provided the protocol and all instructions for safety monitoring of the infusion, according to protocol, with oversight and instruction from the study PI. As per FDA guidance, The study team will attempt to

collect research blood at these visits to be sent to the NIH Clinical Center when possible, to address study outcome measures. During a pandemic affecting patient travel, serial missed research collections will not be considered major study deviations and will be reported to the IRB at the time of continuing review.

Additional reporting requirements to BMS are listed in Appendix A.

17.0 Alternative Therapies

Other treatments available for recurrent glioblastoma include continuing medical therapy (standard radiation and chemotherapy for brain tumors) and surgery to remove recurrent tumor. Furthermore, subjects do not have to participate in this study to receive surgery. They may prefer to receive evaluation and surgical treatment under the care of their own physicians at an outside facility.

18.0 Privacy

All research activities will be conducted in as private a setting as possible.

19.0 Confidentiality

Data collected through this protocol is collected for clinical purposes by clinicians and for research purposes. Medical records with identifiers will be maintained in locked storage in the Surgical Neurology Branch office or at Emory University Hospital. All research data will be de-identified and stored with code numbers as a reference. Electronic records and data will be maintained in computer files which are password-protected and housed on the NINDS network or the Emory University network. Access to all files will be restricted to the Principal Investigator, study coordinators, and the research team. Data collected through patient participation in protocol **03-N-0164** will be shared with this protocol. All team members have completed the OHSRP training.

All collected research samples, including surgical tumor specimens, CSF, microdialysate, and blood will be coded and stored in secured freezers on the NIH campus or at Emory University Hospital. All patients enrolled in this protocol will be assigned a sequential code and all biological samples collected for this patient will be labeled with the patient's code, date of collection, type of sample, sample number, and barcode which will be linked back to relevant sample information in the study laboratory notebooks and Labmatrix.. All study data will be kept on password-protected computers on the NIH campus or at Emory University Hospital. Only study investigators will have access to the stored data and samples, except as noted in the data and sample sharing procedures, above. The study investigators will have access to the code key.

De-identified results from clinical trials will be posted on cctrials.gov

20.0 Conflict of Interest

NIH guidelines on conflict of interest have been distributed to all investigators. Bristol-Myers Squibb, the maker of Nivolumab and BMS-986-016, is providing a drug-only sponsorship for this study. No investigator has a conflict of interest for this study, and no payment or any other benefit, financial or otherwise, will be received by any investigator or the NIH in relation to this trial.

21.0 Technology Transfer

We are currently negotiating a Clinical Trials Agreement for the performance of this study with the drug sponsorship of Bristol-Myers Squibb. A CRADA is not needed, as no funding will be sent directly to the NIH. Coded data will be shared with Bristol-Myers Squibb as a result of this agreement.

Data and samples may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained. Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

22.0 Research and Travel Compensation

The subject will not receive financial compensation for participating in this research study. Enrolled subjects at the NIH will be reimbursed for domestic travel, lodging, and meals according to the NINDS travel policy. Domestic guardian travel and meals will be provided if medically required. Enrolled subjects at Emory University Hospital will not receive reimbursement for travel.

23.0 Definition of Primary and Secondary Data

Primary data are data that are collected specifically for the purposes of the research. Non-interventional research designs based on use of primary data from patients and/or healthcare providers include but are not limited to:

- prospective research (eg, active data collection with active ascertainment of AEs by clinical encounter such as patient interview) and surveys directed proactively to either patients and/or healthcare providers to capture
- information on treatment AEs for a BMS product

Secondary data are data that were originally collected for other purposes (such as patient charts, administrative claims, insurance database, etc).

Such research includes but is not limited to:

- database studies, eg, insurance claims database analyses
- existing surveys, eg, NHANES
- medical chart review studies.

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25.0 Study Calendar

	Before Surgery	Day 1	Qday Day 1 - Day 8	Day 3	Q6h Day 1 - Day 8	Day 8	Day 17 (+/- 2 Days)	Day 31 and every 2 wk. until Progression (+/- 2 Days)	Survival Follow-up (approx.. monthly until death)
Informed consent	X								
Demographics and Medical history	X								
Physical exam (neuro exam, skin exam, vitals, weight, Karnofsky Status)*	X	X	X	X		X	X	X	
CT Head		X							
Chest X-ray	X								
Comprehensive metabolic panel (with creatinine)+ GGT	X	X	X	X		X	X	X	
TSH	X			X			X	X	
CPK				X			X	X	
CRP				X			X	X	
Free T3 and T4	X						X		
Liver function tests (including Tbili, AST and ALT)	X			X			X	X	
CBC with differential	X	X	X	X		X	X	X	
Monitoring for infusion reaction and cytokine release syndrome				X			X		
QAC and QHS accuchecks			X	X					
ACTH and Cortisol**	X						X	X	
MRI Brain with/without contrast	X					X	X	X***	
Biopsy/microdialysis catheter placement		X							
Nivolumab administered				X			X	X	
BMS-986016 antibody administered							X	X	
Research blood sample taken	X	X	X	X		X	X	X	

PK Blood sample taken				X					
EKG and Troponin level****	X						X	X	
Echocardiogram	X						X		
CSF sample taken*****		X	X	X		X			
PK CSF sample taken				X					
Microdialysis sample taken				X	X	X			
Surgical resection/ Microdialysis catheter removal						X			
Bone marrow examination******		X				X			
Adverse event evaluation	X	X	X	X		X	X	X	
Telephone/Medical Record follow-up									X

*Neurological exams will be conducted q2h for the first 24 hours after the research surgical procedure, then q4h while awake for the remainder of the microdialysis monitoring period
**Baseline ACTH and AM Cortisol levels will be obtained, and then further testing will be at the discretion of the treating physicians as clinically indicated
***MRI will be performed every other site visit in follow-up starting at day 17 (every 4 weeks)
****EKG and Troponin level will be taken before each of the first 5 doses of study drugs
*****In addition to daily research samples, CSF will be collected qMonday and Thursday for cell count, protein, glucose, gram stain and culture during inpatient stay as long as the lumbar drain remains in place
******Bone marrow examination is performed at the discretion of the PI

26.0 Consent Forms

Adult subject
Adult assent

27.0 Eligibility Checklist

Inclusion Criteria ***Note that if any box is marked "NO", the subject is not eligible for enrollment.***	**(Yes/No)**
1. 18 years of age or older.	
2. Have recurrent glioblastoma that is amenable to surgical resection.	
3. Agree to undergo brain surgery.	

4. Are eligible for 03-N-0164 "Evaluation and Treatment of Neurosurgical Disorders" protocol	
5. Willing and able to appoint a durable power of attorney.	
6. Are willing to use an effective method of contraception during the clinical study as defined on the consent during the duration of the study and for 24 weeks (for women) or 33 weeks (for men) after the last dose of the study drug.	
Exclusion Criteria ***Note that if any box is marked "YES", the subject is not eligible for enrollment.***	**(Yes/No)**
1. Have a bleeding disorder that cannot be corrected before invasive testing or surgery, or other medical conditions that would make surgery unsafe, such as lung or cardiac disease that would render them unable to tolerate the risk of general anesthesia, or severe immunodeficiency.	
2. Has a known additional malignancy that is progressing or requires active treatment within 3 years of registration. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.	
3. Are pregnant or breastfeeding	
4. Cannot have an MRI scan.	
5. Are claustrophobic	
6. Are not able to lie on their back for up to 60 minutes	
7. Have primary CNS lymphoma.	
8. Has received systemic immunosuppressive treatments, aside from systemic corticosteroids (such as methotrexate, chloroquine, azathioprine, etc) within six months of registration.	
9. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.	
10. Have a significant cardiac history, such as 2 or more MIs OR 2 or more coronary revascularization procedures.	

11. Have abnormal findings on ECG such as prolonged QT interval, T-wave abnormalities or arrhythmia. Abnormal findings on ECG will prompt an evaluation by a cardiologist prior to enrollment in the study	
12. Are currently undergoing treatment with another therapeutic agent.	
13. Has received prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).	
14. Have an ejection fraction <50% on screening echocardiogram	
15. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies) at the time of enrollment.	
16. Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected) at the time of enrollment.	
17. Have an active infection that requires systemic antibacterial, antiviral or antifungal therapy < 7 days prior to initiation of study drug therapy	
18. Have a history of transfer of autologous or allogeneic T cells	
19. Have a history of solid organ or tissue transplants	
20. Have cardiac Troponin T or I > 2x the institutional upper limit of normal at screening	
21. At the time of enrollment, lack consent capacity due to cognitive impairment that would make them incapable of understanding the explanation of the procedures in this study. Cognitive capacity to consent will be determined at the time of enrollment. Patients with mental disorders or those patients who are cognitively impaired yet	
22. Cannot speak English or Spanish fluently	
23. Patients that require dexamethasone > 4 mg/ day or equivalent of steroids	

Appendix A: Bristol-Myers Squibb Adverse Event Reporting:

Adverse Event Reporting

Adverse Events that are routinely collected according to GCP shall be submitted to BMS every three (3) months by the last working day of the third month.
The Adverse Event information required to be sent to BMS is noted in an attached 'Bristol-Myers Squibb Early Asset Investigator Sponsored Research (ISR) Import Plan' which describes the method of collection and submission to BMS via the mailbox: MG-RD-GPVE-PHARMACOVIGILANCE@bms.com
When the file is submitted to BMS, it must be noted the file contains all Non Serious Adverse Events: only adverse events not previously submitted to BMS within the 3 months.

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

Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

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

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

NOTE: (PI determines if this information regarding hospitalizations are considered SAEs and should be included in the protocol. This is supplemental information that is included in BMS-sponsored trials)

The following hospitalizations are not considered SAEs in BMS clinical studies:
a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)

elective surgery, planned prior to signing consent
admissions as per protocol for a planned medical/surgical procedure
routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (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 (applies to oncology protocols)

ADVERSE EVENTS
An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.
The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:
Related: There is a reasonable causal relationship between study drug administration and the AE.
Not related: There is not a reasonable causal relationship between study drug administration and the AE.

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

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

NONSERIOUS ADVERSE EVENT
Nonserious Adverse Events are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [e.g. IND US trial] as part of an annual reporting requirement.
Nonserious AE information should also be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects.

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

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring

after these time periods that is believed to be related to study drug or protocol-specified procedure. The duration of SAE collection should be extended to:
100 days for nivolumab

Worldwide.Safety@bms.comaepbusinessprocess@bms.com
SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours. SAEs must be recorded on BMS or an approved form; pregnancies must be reported on a Pregnancy Surveillance Form.
SAE Email Address: Worldwide.Safety@BMS.com
SAE Facsimile Number: 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.) If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS (or designee) using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

For studies conducted under an Investigator IND in the US, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information. BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at:
http://www.accessdata.fda.gov/scripts/medwatch/.
MedWatch SAE forms should be sent to the FDA at:
MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

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

The following laboratory abnormalities should be documented and reported appropriately:
any laboratory test result that is clinically significant or meets the definition of an SAE
any laboratory abnormality that required the participant to have study drug discontinued or interrupted
any laboratory abnormality that required the subject to receive specific corrective therapy.

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

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.

Potential drug induced liver injury is defined as:
AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)
AND
Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),
AND
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, 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 in accordance with SAE reporting procedures.
Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.
Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form [provided upon request from BMS]
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 nonserious or serious AE, as appropriate, and reported accordingly.
APPENDIX B: INVESTIGATOR BROCHURE FOR NIVOLUMAB (BMS-936558)

APPENDIX C: INVESTIGATOR BROCHURE FOR RELATLIMAB (BMS-986016)

Appendix D: Serious Adverse Reactions in Patients Treated with Relatlimab in Combination with Nivolumab

Table 1-3 Serious Adverse Reactions in Patients Treated with Relatlimab in Combination with Nivolumab (relatlimab monotherapy & relatlimab + nivolumab) Considered Expected for Safety Reporting Purposes (N = 1578)

System Organ Class	Preferred Term	Frequency of All SARs N (%)	Occurrence of Life-Threatening SARs N (%)	Occurrence of Fatal SARs N (%)
Cardiac disorders	Myocarditis	Uncommon 11 (0.69%)	No	No
Endocrine disorders	Adrenal Insufficiency	Uncommon 10 (0.63%)	No	No
	Hypophysitis	Uncommon 5 (0.32%)	No	No
	Thyroiditis	Uncommon 2 (0.13%)	No	No
Gastrointestinal disorders	Colitis	Common 21 (1.33%)	No	No
	Diarrhoea	Uncommon 10 (0.63%)	No	No
	Gastritis[a]	Uncommon 4 (0.25%)	No	No
	Immune-Mediated Enterocolitis[a]	Uncommon 2 (0.13%)	No	No
	Pancreatitis	Uncommon 4 (0.25%)	No	No
General disorders and administration site conditions	Pyrexia	Uncommon 12 (0.76%)	No	No
Hepatobiliary disorders	Autoimmune Hepatitis	Uncommon 8 (0.51%)	No	No
	Hepatitis	Uncommon 4 (0.25%)	No	No

Table 1-3 Serious Adverse Reactions in Patients Treated with Relatlimab in Combination with Nivolumab (relatlimab monotherapy & relatlimab + nivolumab) Considered Expected for Safety Reporting Purposes (N = 1578)

System Organ Class	Preferred Term	Frequency of All SARs N (%)	Occurrence of Life-Threatening SARs N (%)	Occurrence of Fatal SARs N (%)
	Immune-Mediated Hepatitis	Uncommon 3 (0.19%)	No	No
Infections and infestations	Meningitis Aseptic	Uncommon 6 (0.38%)	No	No
Injury, poisoning and procedural complications	Infusion Related Reaction	Uncommon 4 (0.25%)	No	No
Investigations	Alanine Aminotransferase Increased	Uncommon 3 (0.19%)	No	No
	Aspartate Aminotransferase Increased	Uncommon 3 (0.19%)	No	No
	Blood Creatinine Increased[a]	Uncommon 2 (0.13%)	No	No
	Hepatic Enzyme Increased[a]	Uncommon 2 (0.13%)	No	No
	Lipase Increased	Uncommon 3 (0.19%)	No	No
Metabolism and nutrition disorders	Type 1 Diabetes Mellitus	Uncommon 4 (0.25%)	No	No
Musculoskeletal and connective tissue disorders	Myositis[a]	Uncommon 2 (0.13%)	No	No
	Polyarthritis[a]	Uncommon 2 (0.13%)	No	No
Nervous system disorders	Encephalitis Autoimmune[a]	Uncommon 2 (0.13%)	No	No
Respiratory, thoracic and mediastinal disorders	Dyspnoea	Uncommon 2 (0.13%)	No	No
	Immune-Mediated Pneumonitis[a]	Uncommon 2 (0.13%)	No	No
	Pneumonitis	Common 17 (1.08%)	No	No

Table 1-3	**Serious Adverse Reactions in Patients Treated with Relatlimab in Combination with Nivolumab (relatlimab monotherapy & relatlimab + nivolumab) Considered Expected for Safety Reporting Purposes (N = 1578)**			
System Organ Class	**Preferred Term**	**Frequency of All SARs N (%)**	**Occurrence of Life-Threatening SARs N (%)**	**Occurrence of Fatal SARs N (%)**
Skin and subcutaneous tissue disorders	Pemphigoid[a]	Uncommon 3 (0.19%)	No	No

[a]New SARs added for relatlimab in combination with nivolumab in IB version 08.

APPENDIX E: KARNOFSKY PERFORMANCE STATUS

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 their personal needs.
50 – Requires considerable assistance and frequent medical care.
40 – Disabled; requires special care and assistance.
30 – Severely disabled; hospital admission is indicated although death not imminent.
20 – Very sick; hospital admission necessary; active supportive treatment necessary.
10 – Moribund; fatal processes progressing rapidly.
0 – Dead

APPENDIX F: SOCIAL MEDIA LANGUAGE

The below language will be used with social media posts.

Facebook posts:

The National Institute of Neurological Disorders and Stroke is seeking people from across the nation with recurrent glioblastoma to participate in a research study. Domestic travel costs will be reimbursed. This study will evaluate the safety and effects of 2 experimental drugs, nivolumab and relatlimab. The study will look at their effect on the immune response in the brain and on the brain tumor. We will also evaluate the safety of experimental microdialysis catheters. These are thin tubes placed in the brain temporarily to monitor brain immune function. For more information, visit: https://clinicaltrials.gov/ct2/show/NCT03493932

Researchers at the National Institute of Neurological Disorders and Stroke are seeking participants from across the nation for a new research study for people with recurrent glioblastoma. Domestic travel costs will be reimbursed. For more information on this research visit https://clinicaltrials.gov/ct2/show/NCT03493932 or email SNBrecruiting@nih.gov <mailto:SNBrecruiting@nih.gov> #BTSM #endbraincancer

Tweets:

Malignant brain tumors often recur after treatment. This @NIH research study looks at the safety and effect of 2 experimental drugs on brain immune response in recurrent glioblastoma patients: https://clinicaltrials.gov/ct2/show/NCT03493932. Domestic travel costs will be reimbursed for participants

NINDS researchers are studying the safety and effects of 2 experimental drugs on brain immune response in those with recurrent glioblastoma. The NIH will reimburse your domestic travel costs. Learn more about this research here: https://clinicaltrials.gov/ct2/show/NCT03493932 #BTSM #endbraincancer

Learn about a NINDS research study to evaluate the safety and effects of 2 experimental drugs on brain immune response in those with recurrent #braintumors. If you live far from the NIH, your domestic travel costs will be reimbursed: https://clinicaltrials.gov/ct2/show/NCT03493932 #BTSM #endbraincancer

The @NIH brain tumor team is seeking participants for a new research study for people with recurrent glioblastoma: https://clinicaltrials.gov/ct2/show/NCT03493932 We welcome participants from across the US. The NIH will reimburse your domestic travel costs for screening and study participation #BTSM

The @NIH brain tumor team is actively recruiting people from across the nation with recurrent glioblastoma for a research study at the @NIHClinicalCntr: https://clinicaltrials.gov/ct2/show/NCT03493932 The NIH will reimburse your domestic travel costs to Bethesda, MD. #endbraincancer #BTSM