Abbreviated Title: Ph II Nivo IDH-mutant Gliomas

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Title: Phase II trial evaluating Nivolumab in patients with recurrent IDH-mutant gliomas with and without hypermutator phenotype

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Drug Name:	Nivolumab
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Sponsor:	NCI CCR
Manufacturer:	Bristol-Myers Squibb
Supplier:	Bristol-Myers Squibb

Commercial Agents: None

PRÉCIS

Background:

- Glioma is the most common malignant brain tumor. Genes coding for isocitrate dehydrogenases 1and 2 (IDH1 and IDH2), metabolic enzymes, are frequently mutated in gliomas, particularly lower-grade gliomas (LGGs). *IDH1/2* mutation causes a unique tumor biology, including the accumulation of 2-hydroxyglutarate (2-HG), an oncometabolite, which in turn causes genomic hypermethylation and tumorigenesis.
- IDH-mutant LGGs undergo a slow but unremitting progression to higher grade transformation (HT) and eventually become high grade gliomas (HGGs) with a significant increase in the number of somatic mutations. A subset of patients with transformed HGGs develop a hypermutator phenotype (HMP), possibly related, but not limited, to previous treatment with alkylating agents and radiotherapy. The mechanisms of this clinical phenomenon are not fully understood, and no effective treatments are available for the HMP HGGs.
- High tumor mutation burden (TMB) is a characteristic finding in many of the transformed tumors. Furthermore, this increased mutation burden, with commensurate increase in neoantigen expression, may be correlated with a better response to immune checkpoint inhibitor (ICPIs) treatment.
- Nivolumab is a monoclonal antibody that binds to the PD1 receptor and blocks its interaction with PD L1 and PD L2 and subsequently releasing PD 1 pathway mediated inhibition of the immune response, including antitumor immune response.
- The US Food and Drug Administration granted approval to nivolumab for the treatment of unresectable or metastatic melanoma, advanced non-small cell lung cancer, renal cell carcinoma, Hodgkin's lymphoma, recurrent or metastatic squamous cell carcinoma of the head and neck, locally advanced or metastatic urothelial carcinoma, microsatellite instability-high or mismatched repair deficient metastatic colorectal cancer and hepatocellular carcinoma.
- The first randomized clinical trial in glioblastoma with nivolumab (CheckMate-143) was completed in early 2017. Unfortunately, the study didn't meet its primary endpoint of improved overall survival over bevacizumab monotherapy. The objective response rate (ORR) was lower in nivolumab arm than bevacizumab arm. However, the response with nivolumab was more durable. The safety profile of nivolumab was very consistent with what has been observed in other tumor types.

Objective:

- To determine the 6-month progression free survival rate in IDH-mutant gliomas patients with and without HMP in responses to nivolumab treatment.

Eligibility:

- Patients with diffuse glioma, confirmed by NCI Laboratory of Pathology
- Age $\geq$18 years,
- KPS $\geq$60%

- *IDH1* or *IDH2* mutation confirmed by DNA sequencing
- Patients must have TMB status performed at NIH
- Tumor tissue or slides should be available for molecular and immune profiling

Design:

- This study is an open label phase II clinical trial of the immune checkpoint inhibitor, nivolumab, in patients with HMP and NHMP IDH-mutant gliomas.
- Patients with HMP and NHMP will receive nivolumab at a standard dose of 240 mg intravenously every 2 weeks for cycles 1-2, then doses of 480 mg every 4 weeks for cycles 3-16. A maximum of 20 treatments will be given (16 cycles).
- A maximum of 29 patients with IDH-mutant glioma with HMP (Cohort 1) and 30 patients with NHMP (Cohort 2) will be evaluated.
- A Simon's optimal two-stage design will be used to conduct the HMP arm and the NHMP arm independently. For the HMP cohort, in stage I, a total number of 10 patients are accrued. If 9 or more patients progress by 6 months, the cohort will be terminated early; otherwise, additional 19 patients will be accrued in stage II, resulting in a total sample size of 29. Among these 29 patients, if 6 or more patients are progression-free at 6 months, we will claim that the treatment is promising for patients with HMP IDH-mutant gliomas. For NHMP cohort, in stage I, a total number of 15 patients are accrued. If 3 or more patients are progression-free at 6 months, the cohort will move to stage II and additional 15 patients will be accrued in stage II, resulting in a total sample size of 30. Among these 30 patients, if 10 or more patients are progression-free at 6 months, we will claim that the treatment is promising for patients with NHMP IDH-mutant gliomas.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine the 6-month progression free survival rate in IDH-mutant gliomas patients with and without hypermutator phenotype (HMP) in responses to nivolumab treatment.

1.1.2 Secondary Objectives

- To determine the 1-year progression free survival rate and overall survival in IDH-mutant gliomas patients with and without HMP in response to nivolumab treatment.
- To determine whether the neoantigen burden in tumor prior to the treatment is correlated with treatment response.
- To longitudinally evaluate patient reported outcome measures using self-reported symptom severity and interference with daily activities using the MDASI-BT.

1.1.3 Exploratory Objectives

- To determine if a change in immunocompetence defined by peripheral blood mononuclear cell (PBMC) assays and phenotyping over the course of the study treatment correlates with the response and/or treatment-related toxicity.
- Evaluate the immune infiltrates profiles in the tumor microenvironment using quantitative immunofluorescences, digital spatial profiling (DSP) and/or RNA sequencing prior to treatment of nivolumab and correlate with treatment response.
- Measure T cell receptor (TCR) repertoire in PBMC, pre-nivolumab treatment and post every 2 cycles until progression to determine expansion of specific clones and correlation with response.
- Evaluate the epigenetic signatures in tumor samples at baseline and correlate with the mutation burden and treatment response by analyzing DNA methylation by the Infinium Methylation BeadChip.
- Evaluate the genomic features of cell free DNA in the peripheral blood pre-nivolumab treatment cycles 1,2, 3 and at disease progression

1.2 BACKGROUND AND RATIONALE

1.2.1 *IDH* mutation dictates glioma biology

Gliomas are the most common malignant brain tumors and nearly 17,000 people lose their lives to this disease every year. Genes encoding for isocitrate dehydrogenases 1 and 2, *IDH1* and *IDH2*, are frequently mutated in gliomas and they are the most commonly mutated metabolic genes that have been identified in human cancers [1-4]. The mutant IDH1/2 causes loss of its normal function, the production of α-ketoglutarate (α-KG), and the gain of function to produce 2-hydroxyglutarate (2HG) [3, 5]. As a consequence of the mutation, IDH-mutant gliomas are unique phenotypically, genetically and epigenetically [6-8]. Therefore, the *IDH* mutation status was used as a major genetic marker to classify the diffuse astrocytic and oligodendroglia tumors by the most recent WHO Classification of Tumors of the Central Nervous System [9]. Glioma patients with mutant

IDH have better prognosis than those with wild type IDH [2] and some studies have provided the evidence to suggest that IDH-mutant gliomas are more sensitive to chemotherapy [10, 11].

1.2.2 IDH-mutant gliomas accumulate increased mutation burden during higher grade transformation

Despite the fact that patients with IDH-mutant gliomas survive longer, most tumors eventually go through higher grade transformation. In an integrated genomic analysis of the genetic and epigenetic basis for the malignant progression in IDH-mutant gliomas, the non-linear clonal expansion of the original tumors and new oncogenic pathways were identified in the same tumor at the time of malignant transformation [12]. A subset of recurrent tumors was found to be hypermutated and acquired the mutation in mismatch-repair genes. A recent study demonstrated that the mutant IDH1 downregulates the DNA damage sensor ATM by altering histone methylation, leading to impaired DNA repair[13]. As a DNA repair enzyme, α-KG-dependent alkB homolog (ALKBH) is suppressed by 2-HG as a consequence of *IDH* mutation. Cells expressing mutant IDH display reduced repair kinetics, accumulate more DNA damages [14].

Alkylating agents have been used as the major chemotherapy for gliomas, including the IDH-mutant gliomas. Temozolomide (TMZ) has been a commonly used chemotherapy for diffuse gliomas. However, the inherent vulnerability to DNA damage increased the likelihood of DNA damage accumulation in IDH-mutant gliomas. As with all other alkylating agents, TMZ adds a methyl group to the O6 guanine residue (O6-meG), which is primarily repaired in the cells by O6-methylguanine DNA methyltransferase (MGMT)[15]. The limited repair mechanism of O6-meG renders mismatch repair (MMR) pathway status a critical element to determine the fate of the tumor cells, where the intact MMR leads to cell death and loss of MMR function causes mispairing of guanine with thymine and resistance to TMZ[16, 17]. In the absence of MGMT repair and deficient MMR, C>T/G>A signature and TMZ resistance were found. In a mutational analysis, exposure to TMZ can increase the mutation rate at the time of tumor progression [18]. Post-TMZ recurrences had a 39- to 133-fold increase in the mutation rate relative to the treatment-naïve initial low grade tumor, more than 98 % of which are C>T/G>A mutations which are associated with TMZ-induced mutagenesis[19]. In a subset of TMZ-treated recurrent GBM that arose from IDH1-mutant low-grade astrocytoma, hypermutation was identified. The hypermutated tumors were found to acquire mutations in MMR pathway that were not detected in the initial tumors [18] and TMZ was suggested as a predominant source by the mutational signatures [20]. In summary, compromised DNA repair and the common therapeutic agents may contribute to malignant transformation and the development of a hypermutator phenotype in IDH-mutant gliomas.

1.2.3 Cancers with high mutation burden better respond to checkpoint inhibitors

Tumor mutation burden (TMB) can be affected by a variety of causes, including but not limited to the exposure to mutagens, such as TMZ, and deficient MMR. When TMB is high enough, it may be associated with an increased hyper sensitivity to the immune checkpoint inhibitors, potentially due to the expression of immune-reactive neo-antigens in the tumors. This concept has been proven in a variety of refractory cancers [21-23]. Many of the tumors that respond to immune checkpoint inhibitors have deficient mismatch repair (MMR) mechanisms, which leads to 10 to100 times as many somatic mutations as those with proficient MMR [24-26]. Moreover, findings that are consistent with immune response, such as lymphocyte infiltration was found in MMR deficient tumors [27, 28]. The connection between the presence of deficient MMR and better immune response was made, since the somatic mutation can be recognized by the immune system

[29].There has also been an increasing body of evidence to suggest that the cancers exhibiting high TMB, such as bladder cancer, non-small cell lung cancer and melanoma, are responsive to check point inhibitors [30-32]. High mutation and neoantigen burden may also be associated with improved response to immune checkpoint inhibitor in gliomas[33]. Studies of GBM patients with biallelic mismatch repair deficiency (bMMRD), a cancer predisposition syndrome, demonstrated a high mutation burden in their tumors [33, 34]. In this study, authors have demonstrated that all high grade bMMRD tumors are hypermutant, harboring over 100 exon mutations averagely. In the neoantigen analysis, the mean neoantigen burden was seven to 16 times higher than those of immune responsive melanomas, lung cancers, and microsatellite-unstable GI cancers. The case report of a significant responses of recurrent multifocal GBM to nivolumab in two siblings who have bMMRD has provided appealing evidence to theory that checkpoint inhibitor can induce significant clinical responses.

Although the response to immune checkpoint inhibitors in IDH-mutant gliomas with increased mutational burden may differ from these hypermutated gliomas resulted from germline mutation, it is important to investigate this in patients with IDH-mutant gliomas. There are no studies investigating the concept that the tumors with high TMB better respond to immune therapy. The goal for this proposed clinical study is to proof this concept in IDH-mutant gliomas, which overall are immunogenic[35]. For the purpose of this study, we have adopted the same criteria that is used in FOUNDATIONONE™, an FDA-approved next-generation sequencing based assay for identification of genomic alterations within the cancer related genes, to define the hypermutator phenotype (HMP).

For this study, the HMP is defined as 5 somatic mutations/MB detected by CLIA -certified sequencing study at NCI.

1.2.4 Nivolumab is used as an immune therapy for gliomas

Although T cells within the central nervous system act in an antigen-dependent manner, they often have decreased cytotoxic function in the context of tumor [36, 37]. The tumor cell-induced inhibitory effect on T cells is one of important mechanisms. Programmed death-1 (PD-1) has been recognized as a checkpoint protein that is expressed on activated CD4+ and CD8+ T cells, B cells, monocytes, natural killer T (NKT) cells, and dentritic cells (DCs) [38]. B7-H1, which is also called PD-L1, is a co-stimulatory inhibitory molecule that was found to be expressed in malignant glioma [39]. When T cells expressing PD 1 encounter PD L1, T cell functions are subsequently diminished and cause immunosuppressive microenvironment.

Nivolumab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD L1 and PD L2 and subsequently releasing PD-1 pathway mediated inhibition of the immune response, including antitumor immune response. The US Food and Drug Administration granted approval to nivolumab for the treatment of unresectable or metastatic melanoma, advanced non-small cell lung cancer, renal cell carcinoma, Hodgkin's lymphoma, recurrent or metastatic squamous cell carcinoma of the head and neck, locally advanced or metastatic urothelial carcinoma, microsatellite instability-high or mismatched repair deficient metastatic colorectal cancer and hepatocellular carcinoma. The first randomized clinical trial in glioblastoma with nivolumab (CheckMate-143) was completed in early 2017. The purpose of the study was to compare the efficacy and safety of nivolumab administrated alone versus bevacizumab in patients diagnosed with recurrent glioblastoma, and to evaluate the safety and tolerability of nivolumab administered alone or in combination with ipilimumab in patients with different lines of the GBM

therapy. The primary outcome measure was overall survival (OS), and the secondary outcome included overall survival rate, progression-free survival (PFS), and objective response rate (ORR). The result was presented in May 2017. At the time of final analysis, 369 pts were randomized, where 182 received nivolumab and 165 received bevacizumab. At baseline, 40% patients received nivolumab and 43% of patients received bevacizumab required corticosteroids. The OS was 9.8 months with nivolumab and 10.0 months with bevacizumab, and the 12-mo OS rate was 42% in both arms. PFS medians were 1.5 months (nivolumab) and 3.5 months (bevacizumab). Among evaluable patients treated with nivolumab (n = 153) or bevacizumab (n = 156), ORRs were 8% (nivolumab) and 23% (bevacizumab); duration of response medians was 11.1 months (nivolumab) and 5.3 months (bevacizumab). Treatment-related AEs (TRAEs) occurred in 57% (nivolumab) and 58% (bevacizumab) of patients; the most common TRAEs (≥ 10% of patients in either arm; nivolumab vs bevacizumab) were fatigue (21% vs 14%) and hypertension (1% vs 22%). Grade 3–4 TRAEs were reported in 18% (nivolumab) and 15% (bevacizumab) of patients. Serious AEs (all causality) were reported in 46% (nivolumab) and 35% (bevacizumab) of patients; seizure (8% vs 6%) and malignant neoplasm progression (11% vs 7%) were the only serious AEs reported in ≥ 5% of patients in either arm. AEs leading to discontinuation occurred in 10% (nivolumab) and 15% (bevacizumab) of patients. Unfortunately, the study did not meet its primary endpoint of improved overall survival over bevacizumab monotherapy. The ORR was lower in the nivolumab arm than the bevacizumab arm. However, the response with nivolumab was more durable. The safety profile of nivolumab was very consistent with what has been observed in other tumor types. [40]

NRG Oncology Foundation recently completed the phase I study of checkpoint inhibitors anti-CTLA-4, anti-PD-1, the combination in patients with newly diagnosed glioblastoma [41]. In this study, the treatment was started after concurrent chemoradiation therapy, along with adjuvant chemotherapy with TMZ. The primary endpoint was the dose limiting toxicity (DLT) from the start of immune checkpoint inhibitors (ICIs) to 8 weeks after in each arm. A standard up-and-down design was used, with 6 evaluable patients enrolled at a given dose level. The dose level would be declared safe if no more than 1 of 6 had an DLT. Among 31 analyzable patients, treatment was found well tolerated: no reported grade 5 events, 16% of grade 4 events. The study has demonstrated that ICIs including nivolumab are safe and tolerable with similar toxicity profiles noted with other cancers when given with adjuvant TMZ. The results provide necessary safety data justifying the performance of a subsequent trial to test the efficacy of ICIs in this disease.

1.2.4.1 Dosing schedule of nivolumab:

Studies performed in other cancers have demonstrated that after an induction period, the monthly dosing of nivolumab is equally effective but less taxing on patients. The population pharmacokinetics (PPK) and exposure response analyses have been performed to support the use of a nivolumab 240mg Q2W, 360 mg Q3W, and 480 mg Q4W dosing schedule in cancer patients in addition to 3mg/kg Q2W. 240mg Q2W was selected since it is identical to a dose of 3mg/kg for a subject weighing 80 kg, the observed median body weight in nivolumab-treated cancer patients. The Q3W and Q4W dosing schedule allow flexibility of dosing with less frequent visit, which is more convenient for cancer patients.

1.2.5 Rationale for the patient-reported outcome studies

Precedence for measuring “non-therapeutic” endpoints exists in oncology research. For example, Gemcitabine was approved by the FDA partially as a consequence of the decrease in pain reported in pancreatic patients who were treated, not on the basis of survival improvement, which was

modest, at best. There have been efforts in neuro-oncology to evaluate secondary endpoints using validated instruments as an additional indicator of benefit.

The M.D. Anderson Symptom Inventory-Brain Tumor Module allows the self-reporting of symptom severity and interference with daily activities. The MDASI-BT has demonstrated reliability and validity in the adult primary brain and spine tumor patient population. [42, 43] This tool represents a modification of the widely used and validated M.D. Anderson Symptom Inventory, with particular attention to symptoms common in patients with brain and spine tumors respectively.

The MDASI consists of symptoms rated on an 11-point scale (0 to 10) to indicate the presence and severity of the symptom, with 0 being "not present" and 10 being "as bad as you can imagine." Each symptom is rated at its worst in the last 24 hours. Symptoms included on the instrument include those commonly associated with cancer therapies, those associated with increased intracranial pressure, and those related to focal deficits. The questionnaire also includes ratings of how much symptoms interfered with different aspects of a patient's life in the last 24 hours. These interference items include general activity, mood, work (includes both works outside the home and housework), relations with other people, walking, and enjoyment of life. The interference items are also measured on 0 - 10 scales. The average time to complete these instruments is 5 minutes. The MDASI-BT has been translated into multiple languages, but the English language version will be used for this study. In addition, information regarding demographics and treatment history will be collected as part of the larger study and used in this analysis.

The availability of validated instruments provides an opportunity to prospectively assess the impact of treatment, both positive and negative, on patients. The evaluation of symptom burden in this study will assist in finding the best possible treatment with the least toxicity.

Evaluation of the symptom across the scores are an important endpoint for the study. Assessment during the course of treatment may provide important complementary outcome data, which allows the determination of the net clinical benefit of each treatment. The common adverse events associated with nivolumab may result in a measurable effect on patient's well-being and quality of life and those impacts are unlikely to be captured by conventional measures of efficacy such as MR imaging.

1.2.6 Rationale for the correlative studies with biospecimen

The tumor microenvironment contains both tumor cells, which express immune regulatory molecules, and cells with cytolytic capacities. We hypothesize that the immune check point inhibitor conveys the antitumor effects through affecting the whole tumor microenvironmnet, whose profile is essential for the analysis of treatment response. In this study, we plan to collect both blood samples and tumor samples for the purpose of immune monitoring (See details in the Section **5**). The results may provide insights into treatment responses or resistance and potentially lead to the rationale for combined therapies with nivolumab.

1.2.7 Rationale for changing sample number of NHMP cohort

Under the protocol version C (version date 12/09/2021), we performed an interim analysis after a total number of 15 NHMP patients were accrued. 6 patients are progression-free at 6 months. Per protocol, NHMP cohort should proceed to stage II and recruit additional 31 patients. When the characteristics of the 15 patients treated in this cohort were analyzed, 8 of the 15 patients had diagnosis of glioblastoma (WHO grade 4) prior to the study treatments, 6 with anaplastic glioma

(WHO grade 3) and only one with grade II glioma. The distribution of the tumor histology for this cohort was unexpected. The original sample size calculation of the current protocol was based on the expectation of grade III gliomas being the predominant histology, which led to setting the null hypothesis PFS6 being 0.3 (usually used for grade 3 gliomas) and the alternative hypothesis PFS6 being 0.5. As a result, the two-stage design required 6 or more patients among 15 treated patients achieve PFS6 to move to stage II, where additional 31 patients will be enrolled.

In light of the unexpected patient population that were treated in the NHMP cohort, the null and alternative hypotheses, as well as the two-stage design, should be adjusted to reflect the actual patient population. Based on the literature, PFS6 is 0.3 for grade 3 gliomas, and 0.1 for grade 4 glioblastoma. Thus, the updated null hypothesis for PFS6 = (0.1x 8 + 0.3x 6)/ (8 + 6) = 0.186. Assuming 20% improvement as the original design, the alternative hypothesis for PFS6 is 0.386. Employing the Bayesian optimal phase 2 design, the updated 2-stage design becomes: enroll 15 patients, if there are 3 or more patients are progression-free at 6 months, move to stage II and enroll additional 15 patients. Among the total of 30 patients, if 10 or more patients are progression-free at 6 months, we claim that the treatment is promising for patients with NHMP IDH-mutant gliomas. This updated design yields 77.5% power, while controlling one-sided alpha at 0.05.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have recurrent diffuse glioma (histologically confirmed by NIH Laboratory of Pathology) with *IDH1* or *IDH2* mutation (confirmed by DNA sequencing, FoundationOne is preferable for confirmation of mutation, but not necessary).

2.1.1.2 Patients must have tumor specific mutation burden (number of somatic mutations per exome) tested at NIH: Must have either result of tumor mutation burden from the most recent surgical tumor sample or must provide adequate genomic materials of the sample for tumor testing. The tumor tissue (e.g. block or 15 unstained slides) must be available for molecular and immune profiling. Fresh or frozen tumor sample will be used if available, but not mandatory.

2.1.1.3 Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of nivolumab in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

2.1.1.4 Patient must be able to tolerate an MRI study with intravenous gadolinium contrast.

2.1.1.5 Karnofsky ≥60% (**Appendix A**)

2.1.1.6 Patients must have adequate organ and marrow function as defined below:

- Absolute neutrophil count ≥1,500/mcL
- Platelet Count ≥100,000/mcL
- Hemoglobin > 9.0 g/dL (may be transfused to achieve this level)
- BUN ≤ 30 mg/dL and

- Serum creatinine ≤ 1.7 mg/dL
- Total bilirubin (except patients with Gilbert's Syndrome, who are eligible for the study but exempt from the total bilirubin eligibility criterion) ≤ 2.0 mg/dL
- ALT **and** AST ≤ 2.5x institutional upper limit of normal.

2.1.1.7 The effects of nivolumab on the developing human fetus are unknown. For this reason, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation and up to 5 months (women). Should a woman become pregnant or suspect she is pregnant while she is participating in this study, she should inform her treating physician immediately.

2.1.1.8 The patient must be able to understand and be willing to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents.

2.1.2.2 Patients who have a history of receiving immune therapy.

2.1.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to nivolumab.

2.1.2.4 History of severe hypersensitivity reaction to any monoclonal antibody.

2.1.2.5 Prior invasive malignancy (except non-melanomatous skin cancer) unless disease free for a minimum of 3 years prior to initiation of study therapy.

2.1.2.6 Patients with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome or CIDP, myasthenia gravis; systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease (IBD), Crohn's, ulcerative colitis, hepatitis ; and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome. Such diseases should be excluded because of the risk of recurrence or exacerbation of disease.

Of note, patients with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Patients with rheumatoid arthritis and other arthropathies, Sjögren's syndrome and psoriasis controlled with topical medication and patients with positive serology, such as antinuclear antibodies (ANA), anti-thyroid antibodies should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.

2.1.2.7 The patient must not be currently on a corticosteroid dose greater than dexamethasone 1 mg per day or its equivalent.

2.1.2.8 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac

arrhythmia, or psychiatric illness/social situations (within timeframes identified in the bullets below) that would limit compliance with study requirements.

2.1.2.9 Known HIV-positive or acquired immune deficiency syndrome (AIDS) based upon current CDC definition; note, however, that HIV testing is not required for entry into this protocol. The need to exclude patients with AIDS is based on the lack of information regarding the safety of nivolumab in patients with active HIV infection.

2.1.2.10 Pregnant women are excluded from this study because nivolumab's potential for teratogenic or abortifacient effects is unknown. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with nivolumab, breastfeeding should be discontinued if the mother is treated with nivolumab.

2.1.3 Recruitment Strategies

Trial will be posted on www.clinicaltrials.gov, on other NIH websites and on NIH social media forums.

Patients may be enrolled from Natural History Study (NIH 16C0151), which includes all patients who have had their care for brain tumor at NOB. NOB patients are from entire United States.

Furthermore, the investigators plan to contact and network with existing Patient Advocacy Groups serving brain tumor organizations, including American Brain Tumor Association, National Brain Tumor Society.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in section **12.8.2.**

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study screening consent. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

The screening evaluation must be completed within 14 days prior to initiation of study therapy unless otherwise noted:

- Complete medical history and physical examination, including height, weight, vital signs, Karnofsky performance status **(Appendix A:** Performance Status Criteria and neurological exam (**Appendix B**). Height is optional and may be taken at any timepoint during the study, at the discretion of the investigator.

- Laboratory Evaluation
  - Hematological profile: CBC with differential and platelet count;
  - Biochemical profile: electrolytes, BUN, creatinine, AST, ALT, total bilirubin, calcium, phosphorus, albumin, magnesium, uric acid, amylase, lipase;
  - Serum pregnancy test for female participants of childbearing age (in the absence of prior hysterectomy) (24 hours prior to initiation of study therapy);
- MRI of brain study with intravenous gadolinium contrast (within 28 days prior to initiation of study therapy).
- Collection of slides/tumor blocks for molecular and immune profiling. (anytime prior to initiation of study therapy)
- Histologic confirmation (any time prior to initiation of study therapy):
  - Diagnostic confirmation of diffuse glioma by Laboratory of Pathology at NIH
  - Confirmation of *IDH1/2* mutation by DNA sequencing. If patient does not have this test, it can be done at the Laboratory of Pathology at NIH.
  - Tumor specific mutation burden analysis performed at NIH.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825

2.3.1 Screen Failures

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

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a reversible medical conditions, such as kidney dysfunction may be rescreened.

2.3.2 Treatment Assignment Procedures (For registration purposes only):

Cohort

Number	Name	Description
1	HMP	IDH-mutant gliomas patients with hypermutated phenotype (HMP)

2	NHMP	IDH-mutant gliomas patients with non-hypermutated phenotype (NHMP)

Arm

Number	Name	Description
1	Nivolumab	IV Nivolumab

Arm assignment

Subjects in Cohort 1 and 2 will be directly assigned to Arm 1.

2.4 BASELINE EVALUATION

Within 14 days prior to first dose, unless otherwise specified:

- Concomitant Medications
- MRI of the brain study with intravenous gadolinium contrast (within 28 days).

Tests done at screening do not need to be repeated at baseline if performed in designated time frame prior to start of study drug.

- EKG
- Collection of MDASI (For English speaking subjects only)
- Collection of blood for research (See Section **5.1**)

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a phase II study to evaluate the efficacy of treatment with nivolumab in glioma patients with or without HMP.

The study will be conducted in 2 stages.

During stage 1 of the study, 10 patients with HMP and 15 patients with NHMP will be accrued simultaneously into Cohorts 1 (HMP) and 2 (NHMP).

If 9 or more patients with HMP (Cohort 1) progress by 6 months, the Cohort 1 will be terminated early; otherwise, additional 19 patients will be accrued in stage II, resulting in a total sample size of 29.

If 3 or more patients with NHMP (Cohort 2) are progression-free at 6 months, the Cohort 2 will be moved to stage II and additional 15 patients will be accrued, resulting in a total sample size of 30.

All patients will be treated with nivolumab at a flat dose of 240 mg intravenously every 2 weeks (+/- 3 days) for cycles 1 through 2, then at a flat dose of 480 mg every 4 weeks for cycles 3-16. Treatment will be discontinued after 20 treatments given within 64 weeks.

One cycle is 4 weeks (+/- 3 days).

Study assessments presented in the Study Calendar (Section **3.5**).

3.2 DRUG ADMINISTRATION

Treatment will be routinely administered on an outpatient basis. Nivolumab will be administered through IV infusion over 30-60 minutes at a flat dose of 240 or 480 mg. See Section **14.1.5** for more details about study agent administration.

Vital signs will be measured within 1 hour before and after drug infusion.

If the eligible patients receive re-radiation immediately after pathology proving recurrent disease, additional proof of disease progression after re-radiation therapy is needed prior to the study treatment.

3.3 DOSE MODIFICATIONS

There are no planned dose reductions for nivolumab.

The tables below outline the criteria for delaying (then restarting) or stopping treatment by toxicity type.

Treatment will be discontinued if AE is not resolved for 4 weeks.

Skin Rash and Oral Lesions	**Management**
Grade 1	Continue treatment. No change in dose *.
Grade 2	Hold* until 1≤ Grade resolved. Consider steroid treatment > 5-7 days Resume at same dose level.
Grade 3	Hold* until ≤ Grade 1. Resume at same level at investigator discretion
Grade 4	Off protocol therapy
*Patients with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphigoid. Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.	
Recommended management: See AE management guidelines (**Appendix C**)	

Liver Function AST/ALT/T. Bili *	**Management**
Grade 1	Continue treatment
Grade 2	Hold until less than or equal to Grade 1. Resume at same dose level. Consider steroid treatment if persists > 5-7 days or worsens.

Liver Function AST/ALT/T. Bili *	**Management**
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Continued treatment of active immune mediated hepatitis may exacerbate ongoing inflammation. Holding drug to evaluate LFT changes and early treatment are recommended. LFT changes may occur during steroid tapers from other events and may occur together with other GI events including cholecystitis/pancreatitis.	
Recommended management: See Hepatic AE management algorithm (**Appendix C**)	
* Total bilirubin not used for determining AEs in patients with Gilbert syndrome.	

Renal Function Serum Creatinine	**Management**
Grade 1	Continue treatment.
Grade 2	Hold until≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤Grade 1. Resume at same dose level.
Grade 4	Off protocol therapy
For Grade 2 or greater, consider renal biopsy. Corticosteroids may be required for Grade 2/3, high dose for grade 4.	
Recommended management: see Renal AE management algorithm (**Appendix C**)	

Diarrhea/ Colitis	**Management**
Grade 1	Diarrhea/asymptomatic colitis, continue treatment at PI discretion.
Grade 2	Hold until ≤Grade 1 or baseline. Consider steroid treatment if persists > 5-7 days.
Grade 3	Off protocol therapy.
Grade 4	Off protocol therapy.
Patients with grade 2 symptoms but normal colonoscopy and biopsies may be retreated after resolution. Patients with grade 2 persistent symptoms greater than 14 days who require steroids should be taken off study treatment. Please evaluate pituitary function prior to starting steroids if possible without compromising acute care. Evaluation for all patients for additional causes includes *C. diff*, acute and self-limited infectious and foodborne illness, ischemic bowel, diverticulitis, and IBD.	
Recommended management: see GI AE management Algorithm (**Appendix C**).	

Pancreatitis Amylase/Lipase	**Management**
Grade 1	Continue treatment if asymptomatic.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level if asymptomatic. Asymptomatic Grade 2 is defined as elevation of both amylase and lipase.

Pancreatitis Amylase/Lipase	**Management**
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Patients may develop symptomatic and radiologic evidence of pancreatitis as well as DM and DKA. Lipase and/or amylase elevation may occur during the period of steroid withdrawal and with other immune mediated events or associated with colitis, hepatitis, and patients who have asymptomatic lipase and/or amylase elevation typically have self-limited course and may be retreated. For treatment management of symptomatic pancreatitis please follow the Hepatic Adverse Event Management Algorithm (**Appendix C**).	

Pneumonitis	**Management**
Grade 1	Hold dose pending evaluation and resolution to baseline including baseline pO2. Resume; no change in dose after pulmonary and/or ID consultation
Grade 2	Hold dose pending evaluation and resolution to baseline including baseline pO2. Resume; no change in dose after pulmonary and/or ID consultation if lymphocytic pneumonitis is excluded. Off study if grade 2 symptoms do not improve or worsen after 2 weeks of steroids and dose delay.
Grade 3	Off protocol therapy.
Grade 4	Off protocol therapy.
Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for patients who do not respond to antibiotics and have no causal organism identified including influenza. Most patients with respiratory failure or hypoxia will be treated with steroids. Bronchoscopy may be required and analysis of lavage fluid for lymphocytic predominance may be helpful. Patients with new lung nodules should be evaluated for sarcoid like granuloma. Please consider recommending seasonal influenza killed vaccine for all patients.	
Recommended management: See Pulmonary Adverse Event Management Algorithm (**Appendix C**).	

Other GI Nausea - Vomiting	**Management**
Grade 1	No change in dose.
Grade 2	Hold pending evaluation for gastritis duodenitis and other immune adverse events or other causes. Resume at same dose level after resolution to ≤ Grade 1.
Grade 3	Hold pending evaluation until ≤ Grade 1. Resume at same dose level. If symptoms do not resolve within 7 days with symptomatic treatment patients should go off protocol therapy
Grade 4	Off protocol therapy.

Other GI Nausea - Vomiting	**Management**
Patients with grade 2 or 3 N-V should be evaluated for upper GI inflammation and other immune related events.	

Fatigue	**Management**
Grade 1	No change in dose.
Grade 2	No change in dose
Grade 3	Hold until ≤ Grade 2. Resume at same dose level
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, and hepatic, or muscle (CPK) inflammation	

Neurologic events	**Management**
Grade 1	Hold dose pending evaluation and observation. Resume with no change in dose.
Grade 2	Hold dose pending evaluation and observation. Hold until ≤ Grade 1.* Off protocol therapy if treatment with steroids is required. Resume at same dose level for peripheral isolated n. VII (Bell's palsy)
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
*Patients with any CNS events including aseptic meningitis, encephalitis, or myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), GB syndrome, myasthenia gravis should be off protocol therapy.	
Recommended management: See Neurologic Adverse Event Management Algorithm (**Appendix C**)	

Endocrine Hypophysitis Adrenal Insufficiency	**Management**
Grade 1	Asymptomatic TSH elevation *. Treatment may continue while having endocrine evaluation. endocrine consult is recommended.
Grade 2	Hold until patients are on a stable replacement hormone regimen. If treated with steroids patients must be stable off steroids for two weeks. Resume at same dose level.
Grade 3	Off protocol therapy
Grade 4	Off protocol therapy
Note all patients with symptomatic pituitary enlargement, exclusive of hormone deficiency, but including severe headache or enlarged pituitary with enhancement on MRI should be considered grade 3 events. Isolated thyroid or testosterone deficiency may be treated as grade 2 if there are no other associated deficiencies and adrenal function is monitored.	

Endocrine Hypophysitis Adrenal Insufficiency	**Management**
Please evaluate pituitary function before beginning steroid therapy or replacement therapy of any kind. *Note patients with thyroiditis may be retreated on replacement therapy. Patients must be evaluated to rule out pituitary disease prior to initiating thyroid replacement.	
Recommended management: See Endocrine Management Algorithm (**Appendix C**)	

Fever	**Management**
Grade 1	No change in dose.
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold until ≤ Grade 1. Resume at same dose level.
Grade 4	Off protocol therapy
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever.	

ALL OTHER EVENTS: NOT AGENT RELATED OR AGENT RELATED NON-IMMUN RELATED	**Management**
Grade 1	No change in dose
Grade 2	Can continue treatment at PI discretion.
Grade 3	Hold until ≤ Grade 1 continue at investigator discretion with the following exception: Grade 3 lymphopenia or asymptomatic amylase or lipase does not require dose delay
Grade 4	Off protocol therapy, with the following exceptions: • Grade 4 neutropenia ≤ 7 days • Grade 4 lymphopenia or leukopenia or asymptomatic amylase or lipase • Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset • Grade 4 drug-related endocrinopathy adverse events, such as, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents,

ALL OTHER EVENTS: NOT AGENT RELATED OR AGENT RELATED NON-IMMUN RELATED	**Management**
	respectively, may not require discontinuation after discussion with and approval from the BMS Medical Monitor.
Recommended management: As clinically indicated	

ALL OTHER EVENTS: IMMUNE RELATED	**Management**
Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1 OR baseline. When resolved or following steroids, resume at same dose level. • Exception: Nivolumab should be discontinued for any grade 2 drug-related uveitis, eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
Grade 3	• Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality, not associated with underlying organ pathology, that does not require treatment except for electrolyte replacement OR hormone/steroid replacement **does not** require treatment discontinuation. • Any grade 2 or 3 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment requires treatment discontinuation
Grade 4	Off protocol therapy, with the following exceptions: • Grade 4 neutropenia ≤ 7 days • Grade 4 lymphopenia or leukopenia or asymptomatic amylase or lipase • Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset • Grade 4 drug-related endocrinopathy adverse events, such as, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents,

ALL OTHER EVENTS: IMMUNE RELATED	**Management**
	respectively, may not require discontinuation after discussion with and approval from the BMS Medical Monitor.
Recommended management: As clinically indicated.	

3.4 QUESTIONNAIRES

MDASI-BT will be completed within 14 days prior to initiating treatment (baseline) and at the time of each imaging study but prior to the patient being informed of the imaging results.

Patients will be asked to continue complete MDASI-BT after treatment discontinuation every 6 months until they are off the clinical trial. This instrument will be available electronically, so patients do not need to come to NIH to complete it after treatment discontinuation.

The MDASI-BT will be completed only by the patient, unless changes in vision or weakness make this difficult. If this occurs, then the caregiver or research assistant may read the questions to the patient or assist with marking the severity number or score as described by the patient. A patient caregiver may complete the questionnaires as a patient-preference proxy if the patient's deficits preclude self-report however, this must be done at every assessment from baseline to end of treatment.

The average time to complete this instrument is 5 minutes. Only for English speaking patients.

3.5 STUDY CALENDAR

Procedure	**Screening**	**Baseline[1]**	**Cycles (28+/-3 days)**			**28 (+/-7 days) Day FU[11]**	**Long Term FU[10, 11]**
			1-2		**3-16**		
			Days[1]		**Days[1]**		
			1	**15**	**1**		
Nivolumab[2]			X	X	X		
Medical History	X						
Confirmation of Pathology and tumor mutation burden	X						
Height[14]	X						
Physical exam, neurological exam, KPS	X		X		X	X	X
Weight[12]	X		X		X		
Vital signs	X		X[3]	X[3]	X[3]	X	
EKG		X					
CBC with differential [4]	X		X		X	X	
Biochemical profile [4, 5]	X		X		X	X	

Procedure	**Screening**	**Baseline[1]**	**Cycles (28+/-3 days)**			**28 (+/-7 days) Day FU[11]**	**Long Term FU[10, 11]**
			1-2		**3-16**		
			Days[1]		**Days[1]**		
			1	**15**	**1**		
Fasting AM cortisol, TSH			X		X	X	
Serum pregnancy test [6]	X		X		X		
Tumor evaluation (MRI)[7]	X	X	X		X		X
Concomitant Medications		X	X	X	X	X	
Adverse events		X	X	X	X	X	X
Research blood for immune monitoring, cell free DNA and TCRseq [8]			X		X		X
Collection of archival tumor sample for research studies	X						
Collection of tumor sample							X[13]
MDASI-BT Questionnaire [9]		X	X		X		X
Phone call or e-mail for survival						X	X

[1] Tests done at screening or baseline do not need to be repeated on baseline or Day 1 of Cycle 1 if performed in designated time frame prior to start of study drug. Time window +/- 3 days is allowed for Days 1 and 15.

[2] 240 mg intravenously on days 1 and 15 during cycles 1 through 2, then doses of 480 mg on day 1 of cycles 3-16.

[3] See section **3.2** for vital signs schedule on days of drug infusion.

[4] During treatment cycles, labs will be obtained within 72 hours prior to dosing

[5] Biochemical profile: electrolytes, BUN, creatinine, AST, ALT, total bilirubin, calcium, phosphorus, albumin, magnesium, uric acid amylase and lipase

[6] For women of child-bearing potential only. Serum pregnancy test must be performed 24 hours prior to Nivolumab initiation and within 24 hours of day 1 of every cycle thereafter. If Nivolumab is initiated within 24 hours after the screening test, pregnancy test does not have to be repeated before the cycle 1 treatment.

[7] MRI of brain with gadolinium at screening/baseline and every 8 (+/- 7 days) weeks after beginning of treatment and at 60 (+/- 14 days) day follow up visit after last dose of Nivolumab. Note: if treatment was discontinued because of progression, the 60 days imaging is not necessary. Subjects taken off treatment before completion of 1 year of treatment and without PD at the 28-Day Safety Follow-up visit will be followed till disease progression (MRI scans every 8 (+/- 7 days) weeks with the first assessment 8 weeks after the previous tumor assessment) or 1 year after start of treatment whatever comes first.

[8] Blood samples will be drawn prior to the initiation of treatment of cycle 1, 2, 3, 5, 7, 9, 11, 13, 15 or until disease progression, whatever comes first. Blood samples will be collected at treatment discontinuation and if patient undergoes surgical procedure after treatment discontinuation, blood samples will be collected for analysis. Also, at the 60 (+/- 14 days) day follow up visit.

[9] At baseline and then within 7 days prior to each imaging study. After disconnection of treatment should be completed every 6 months. MDASI-BT may be completed electronically. MDASI completed within 14 days prior to treatment will be considered baseline.

[10] Follow up visits are planned to be performed at 60 (+/- 14 days) and 100 (+/- 14 days) days after the last dose of Nivolumab to evaluate patient's safety. After this visit, subjects will be followed every 6 months (± 1 month) for survival by phone call or e-mail. Note: Subjects taken off treatment before completion of 1 year of treatment and without PD at the 28-Day Safety Follow-up, will be invited for MRI evaluations every 8 (+/- 7 days) weeks until PD or 1 year after start of treatment whatever comes first.

[11] If subjects are unable or not willing to come to NIH for follow up visits, they will be followed by phone call or e-mail for AEs and survival.

[12] Weight needs to be recorded once per cycle of infusion.

[13] If patient undergoes surgical procedure after treatment discontinuation, tumor tissue will be collected for analysis. Tumor sample obtained at disease progression, if available.

[14] Optional. Height may be taken at any timepoint during the study, at the discretion of the investigator.

3.6 COST AND COMPENSATION

3.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by their insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.6.2 Compensation

Participants will not be compensated on this study.

3.6.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 100 days following the last dose of study therapy.

3.7.1 Criteria for Removal from Protocol Therapy

- Completion of protocol therapy
- Progressive disease (See **Appendix D** for treatment discontinuation guidance)
- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in Section **3.3**
- PI discretion
- Positive pregnancy test
- Patient becomes unable to tolerate an MRI study with intravenous gadolinium contrast.

3.7.2 Off-Study Criteria

- Participant requests to be withdrawn from study
- Death
- PI discretion
- Patient becomes lost to follow-up
- PI decision to close the study
- Screen failure

3.7.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 visits scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit for 30 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

All supportive therapy for optimal medical care will be given during the study period at the discretion of the attending physician(s) within the parameters of the protocol.

No investigational or commercial agents or therapies other than those described above may be administered with the intent to treat the patient's malignancy.

4.1 ALLOWED MEDICATIONS

- *Anticonvulsants*: Anticonvulsants may be used as clinically indicated. Doses at study entry and at specific time points of the treatment must be recorded. **NOTE**: The use of hepatic cytochrome p450 enzyme inducing anticonvulsants (EIAEDs) are permitted.

- *Corticosteroids*: Corticosteroid use should be avoided whenever possible because of the established inhibition of the treatment-induced immune response. However, corticosteroids may be administered to reverse neurologic dysfunction secondary to peritumoral edema at the treating physician's discretion. Furthermore, in cases of significant immune-related toxicities, corticosteroid use may be required as determined by the specific adverse event treatment algorithms provided in **Appendix C**. A maximum use of physiologic doses of corticosteroids are mandated as an eligibility criterion at study entry and if administered, the study entry dose and any subsequent corticosteroid use with the specific dosing schedule and indication for use must be recorded.

- *Antiemetics*: Prophylactic antiemetics may be administered at the treating physician's discretion.

- *Pneumocystis Carinae Prophylaxis*: Patients with severe lymphopenia are at an increased risk for opportunistic infections. Patients with a lymphocyte count < 500/mm^3 should have CD4 quantification. If the CD4 is < 200, then prophylaxis is recommended to continue and the CD4 should be quantified on a monthly basis. If the lymphocyte count is ≥ 500 or the CD4 is > 200, then prophylaxis can be stopped.

- *Antidiarrheals*: Antidiarrheal regimens are allowed if used in accordance with the guideline provided in the algorithm in **Appendix C**. Diarrhea is a frequent toxicity associated with nivolumab so strict adherence to the guidelines is required.

4.2 PROHIBITED THERAPIES

- Other investigational drugs.
- Any cancer therapy other than nivolumab while patient is on the study.
- Stereotactic boost radiotherapy or other forms of local treatment such as the Gliadel wafer.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION

Test/assay	**Volume blood**	**Type of tube[1]/ tissue sample**	**Collection point**	**Location of specimen analysis**
IDH1 or IDH2 mutation by DNA sequencing (if FoundationOne result is not available)		Archival tissue samples	Screening	Laboratory of Pathology
Whole exome sequencing for tumor mutation burden (if was not done on another protocol in NIH), neoantigen analysis		Archival tissue samples Tumor sample obtained at disease progression, if available	Screening Disease progression	Laboratory of Pathology or Genetics Branch
RNA sequencing		Archival tissue samples Tumor sample obtained at disease progression, if available	Baseline Disease progression	Laboratory of Pathology or Genetics Branch
DNA methylation analyses		Archival tissue samples	Baseline	Laboratory of Pathology

Test/assay	**Volume blood**	**Type of tube[1]/ tissue sample**	**Collection point**	**Location of specimen analysis**
		Tumor sample obtained at disease progression, if available	Disease progression	
Quantitative immunofluorescence or digital spatial profiling (DSP)		Archival tissue samples Tumor sample obtained at disease progression, if available	Baseline Disease progression	NOB Lab
Flow cytometry to characterize intra-tumor immune cells and microenvironment		Fresh or frozen tumor sample	If available	NOB Lab
TCRseq		Fresh or frozen tumor sample	If available	Laboratory of Pathology or Genetics Branch
Immune monitoring, including TCRseq Cell free DNA Cytokine profiling	Blood, 48mL (8mL/tube X6 tubes) for pre-cycle 1,2, 3 and at disease progression Starting pre-cycle 5, 32 ml (8mL/tube X4 tubes).	CPB/CPT™ x 4 and Streck tube x2 CPT x2 Streck x2	See study Calendar **3.5**	NOB Lab Trepel Lab Genetics Branch

Test/assay	Volume blood	Type of tube[1]/ tissue sample	Collection point	Location of specimen analysis
	60 Day Follow Up	6 Tubes: CPT x4 Streck x2		
	For pre-cycle 1,2,3 (6 tubes): NOB lab will distribute 2 tubes to the Trepel Lab, 2 tubes to NOB lab and 2 Streck tubes to NOB . Starting pre-cycle 5 (4 tubes): 2 CPT tubes t and 2 Streck tubes to NOB At time of disease progression and at 60day FU, NOB lab will distribute 2 CPT tubes to the Trepel Lab, 2 CPT tubes and 2 Streck tubes to NOB lab			
Whole exome sequencing of germline mutations	Blood, 3 mL Or Saliva (per collection kit)	Sodium citrate tube for WES or Saliva Collection Kit	Refer to Study Calendar **3.5**	Laboratory of Pathology or Genetics Branch

1. Tubes/media may be adjusted at the time of collection based upon availability with permission of PI or research lab.

5.2 TUMOR TISSUE CORRELATIVE STUDIES:

In this study patients must provide documentation or tissue samples to confirm eligibility at screening: glioma by NIH Laboratory of Pathology, IDH1/IDH2 mutational status by sequencing and tumor mutation burden, performed at NIH.

The archival tumor tissue material will be utilized to perform additional research experiments including whole exome sequencing, RNA Sequencing, DNA methylation analyses and quantitative immunofluorescence. The WES, RNAseq and DNA methylation will also be repeated if samples are available.

If patient undergoes surgical procedure prior to the study treatment, or after treatment discontinuation, we will ask patients to provide fresh frozen tissue if available to characterize intra-tumor immune cells and microenvironment and evaluate T cell receptor repertoire.

Tissue specimens will be sent for barcoding and initial storage to Laboratory of Pathology (See Section **5.5.2**).

5.2.1 Whole exome sequencing (WES) and RNA sequencing

DNA and RNA will be extracted from the formalin-fixed paraffin embedded (FFPE) tumor tissue. WES will be performed to determine mutational burden, neoantigen analysis, and somatic

mutations which may be correlated with immunologic response or treatment-related toxicity. RNA can be applied to gene expression profiles, from which CIBERSORT, a method for characterizing cell composition of complex tissue for immune infiltrates profile [44]. This will be done with RNA sequencing.

5.2.2 DNA methylation analysis

TCGA studies in LGG and GBM have demonstrated that *IDH* mutation status was the primary driver of methylome and transcriptome clustering [45]. In IDH-mutant gliomas, three epigenetic subtypes have been defined: 1) the1p/19q co-deletion (Codel) group, 2) G-CIMP low group, including IDH-mutant-non-codel gliomas with low genome-wide DNA methylation, and 3) G-CIMP high group, including IDH-mutant-non-codel gliomas with high genome-wide DNA methylation. More importantly, The G-CIMP low group was associated with significantly worse survival as compared to the G-CIMP high group. The survival of the G-CIMP high group is as favorable as the Codel group, which was considered to have the best prognosis. This evidence suggests that global DNA methylation status of IDH-mutant glioma is closely associated with clinical outcome, which makes the DNA methylation analysis a valuable test for this study of IDH-mutant glioma patients.

5.2.3 Quantitative immunofluorescence and digital spatial profiling (DSP)

Quantitative immunofluorescence and digital spatial profiling can be used for the spatial location of immune infiltrate in the tumor microenvironment using FFPE slides or fresh frozen tissue.

5.2.4 Intra-tumor immune cells and microenvironment

Comprehensive flow cytometry staining panel using validated antibodies will be performed to characterize intra-tumor immune cells and microenvironment using fresh or frozen tumor sample, if available.

5.2.5 Sequencing of TCR repertors (TCRseq)

Immune system is important for tumor survellience and its local anti-tumor potential highly rely on the tumor infiltrating lymphocytes (TILs), particularly the repertoir of T-cell receptors (TCR) and the manipulation of the T cells that infiltrate gliomas [46]. The ability to mount an adaptive immune response relies on these T cell reeptors, which determine the functional activation and clonal expansion for the T cells. High throughout sequencing of TCR repertors (TCRseq) allowing the repertoire profiling [47, 48]. When we correlate the treatment response with tumor neoantigen burden in patients receiving nivolumab, information of the tumor microenvironment prior to the treatment, more specifically, the capacity of adequately stimulated T cells to recognize tumor antigens and respond locally is essential. The information can be obtained by performing TCRseq using the fresh frozen tumor tissue prior to the treatment of immune checkpoint inhibitor starts. Collection of fresh frozen tissue is not required in this study, but this test can be done if the tissue can be provided by the patient as described in section **5.1**.

5.3 BLOOD CORRELATIVE RESEARCH:

Blood specimens will be sent for barcoding and initial storage to Blood Processing Core (See section **5.5.1)**

5.3.1 Immune monitoring

Changes in absolute lymphocyte count (ALC) will be monitored before and after nivolumab to

determine how PD-1 blockade alters ALC in relation to outcome as a predictive biomarker. Prolonged lymphopenia (ALC <1,000 µL) has been found to a negative marker of response to anti-CTLA-4[49, 50] but this is not known with anti-PD-1.

There are two IFN-γ ELISPOT assays that quickly determine whether PBMC samples are immunocompetent to mount $CD8^+$ and $CD4^+$ T-cell responses in vitro that have been validated for use when multiple blood samples are collected and analyzed over a protracted period of time (months or years). The tests measure the ability of the PBMC to react against a series of common recall antigens. For $CD8^+$ T-cell responses, there is an assay that utilizes a 43-peptide recall antigen pool (containing different viral recall antigen peptides from EBV, flu, and CMV) that can measure responses in over 90% of patients regardless of their HLA class I sub-type. A similar ELISPOT approach has been developed to measure patient $CD4^+$ T-cell immunocompetence using recall antigen responses against a chemically inactivated tetanus toxoid (TT) protein and *Candida albicans* extract, 2 agents that essentially all humans have been exposed to naturally or through vaccination [51, 52].

NK cells are critical in helping to initiate adaptive anti-tumor responses and are also key targets of immunosuppressive pathways such as TGF-beta. An intracellular staining flow cytometry assay called "phospho-flow" that measures the tyrosine phosphorylation of STAT1 and STAT5 in response to type I interferon and IL-15, two key NK cell activators will be used to monitor whether the patient's overall immune competence is changing during and following therapy with nivolumab.

Comprehensive flow cytometry staining panel using validated antibodies will be performed to phenotype lymphocytes. This flow cytometry panel includes: (1) FACS phenotyping for $CD4^+$ and $CD8^+$ naïve (T_N), central memory (T_{CM}), effector-memory (T_{EM}) and terminally differentiated effector (T_{TDE}) cells [53, 54]; (2) Detection of antigen-specific $CD8^+$ T cells using HLA class I tetramers; (3) Staining for suppressor cell subsets such as $CD4^+$ T-regulatory cells and myeloid derived suppressor cells (MDSC) subsets; (4) Identification of APC subsets (pDC, mDC, B cells, macrophages) and (5) Track changes in $CD4^+ICOS^+$ effector T cells and $ICOS^+$ Tregs, two $CD4^+$ subsets that have emerged to be biomarkers in anti-CTLA-4 and IL-2 therapy for melanoma[55, 56].

Immune subset analysis is going to be performed in the first 3 cycles to explore the early indicators of response form the peripheral blood sample. Depending on viable PBMC number, the following immune subsets will be assessed using multiparameter flow cytometry including but not necessarily limited to CD8+ T-cells, CD4+Foxp3- T-cells, Tregs, NK cells, monocyte subsets, MDSC and/or DC subsets. Assessment will include functional markers, i.e. PD-1, Tim-3, CTLA-4, PD-L1 and/or HLA-DR.

Multiplex cytokine measurements will be performed using the Luminex-100 and the Meso Scale Discovery (MSD) Systems. This will allow us to establish patterns of cytokine/chemokine secretion in correlation to patient response rather than single-parameter comparisons. Thus, not only the amount of a particular cytokine secreted, but also how many cytokines are secreted and in what combination, can be determined during a T-cell response. Serum cytokine profiles will be longitudinally assessed using this platform with particular focus on TGF-β, galectin-3, IFN-γ, IL-2, IL-6, IL-12, IL-10, and IL-23 modulation. This will also allow us to define the type of response (e.g. Th1, Th2, Tc1, Tc2, Treg) generated by the various treatments.

Blood specimen will be obtained for the purpose of determining immunocompetence of T-cell and NK cell function. Cytokine profile will be measured by Luminex-100 and MSD system. Blood samples will be drawn at time points indicated in Study Calendar (Section **3.5**).

5.3.2 TCRseq

In the peripheral blood sample, peripheral blood mononuclear cells (PBMC) can be used for TCRseq to determine the T cell clonal expansion in response to the immunotherapy. The TCR repertoire profiling using peripheral blood throughout the treatment enables the identification of expansion of specific clones and longitudinal monitoring of systemic response to therapy. This may provide guidance for rational combination of immunotherapies. Immunocompetency of T and NK cell can be tested using PBMC and it may provide the insight of treatment response to immune check point inhibitors.

The TCRseq from peripheral repertoire using PBMC will be measured in blood samples collected at time points indicated in Study Calendar **3.5** to determine expansion of specific clones and correlate with systemic response to the immune treatments.

5.3.3 DNA evaluation

A small aliquot of PBMCs will be stored for subsequent extraction of DNA for evaluation of the patient's genome. These data will be used for comparison with tumor DNA and for studies of single nucleotide variations to search for predictors of response and/or nivolumab toxicity.

5.4 SAMPLE PROCESSING

5.4.1 Trepel Lab blood sample processing

NOB Study team will contact the Trepel Lab, Developmental Therapeutics Branch, NCI (Jane Trepel- trepel@helix.nih.gov; Min-Jung Lee- leemin@mail.nih.gov; Akira Yuno – akira.yuno@nih.gov and Sunmin Lee - lees@pop.nci.nih.gov) when the patient is scheduled. The NOB lab designee is going to call Trepel lab as soon as the blood is drawn at 240-760-6330 and a member of the Trepel lab will come to pick up the immune subsets blood samples. Blood sample will be kept at ambient temperature. Members of the lab will enter the samples in a secure patient database and process the samples.

5.4.2 All other blood sample processing

All other research blood, including immune monitoring, TCRseq and WES will be picked up by the NOB lab designee, delivered to NOB lab for processing. The sample for immune monitoring will be barcoded by Susie Ahn and stored in NOB lab as described in **5.5.5**. The sample for TCRseq will be delivered to Song Young at Genetic Branch (TEL: 240-760-7418) for processing, barcoding and storage, as described in **5.5.3.**

5.4.3 Tumor tissue processing

All tumor tissue block and slides will be collected by the study team and sent to Genetic Branch or Laboratory of Pathology for WES, RNA sequencing and DNA methylation, as described in **5.5.2** and **5.5.3**; to NOB laboratory for quantitative immunofluorescence, as described in **5.5.5**. If patients undergo surgeries, Susie Ahn or NOB lab designee will be paged to pick up the fresh or frozen tissue, which was be processed and stored in NOB for flow cytometry and genetic Branch for TCRseq, as described in **5.5.5** and **5.5.3**, respectively.

5.5 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through the Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.5.1 Procedures for Storage of Specimens in the NOB

Patient samples, collected for the purpose of research under IRB approved protocols conducted by NOB, may be archived in the NOB laboratory. All data associated with archived clinical research samples is entered into the NCI Labmatrix database. These databases are stored on the NCI group drive in the Clinical Service folder. Access to this folder is limited to NOB research staff, requiring individual login and password..

The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn/collected, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, marrow, tissue) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI clinical records. All received samples will receive a unique bar code number, which will be added to the sample NCI Labmatrix database. Only this bar code will be recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the NCI Labmatrix database.

Samples are stored in freezers at -80^{0}C (sera, plasma, tissue samples) or under liquid nitrogen (cells), according to stability requirements. These freezers are located onsite at the NOB laboratory. Access to samples from a protocol for research purposes will be by permission of the Principal Investigator.

5.5.2 Procedures for Storage of Tissue Specimens in the Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly, and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissues are stored for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.5.3 Procedures for Storage of Specimens in the Genetic Branch under Supervision of Dr. Javed Khan

Patient samples, collected for the purpose of research under IRB approved protocols conducted by CGB, may be archived in the CGB laboratory. All data associated with archived clinical research samples is entered into the NCI Labmatrix database. These databases are stored on the NCI group drive in the Clinical Service folder. Access to this folder is limited to CGB research staff, requiring individual login and password. .

The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn/collected, treatment cycle/post-transplant time point, cell source (e.g. peripheral blood, marrow, tissue) as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI clinical records. All received samples will receive a unique bar code number, which will be added to the sample NCI Labmatrix database. Only this bar code will be recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the NCI Labmatrix database.

Samples are stored in freezers at -80^0 C (sera, plasma, tissue samples) or under liquid nitrogen (cells), according to stability requirements. These freezers are located onsite at the CGB laboratory. Access to samples from a protocol for research purposes will be by permission of the Principal Investigator.

5.5.4 Procedures for Storage of Specimens for Laboratory of Jane Trepel

Samples will be processed immediately by the Trepel laboratory. Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality and patient confidentiality. Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. Specimen labels will indicate protocol number, order in which the patient enrolled on the trial, type of sample, collection time, and total volume collected, as appropriate. The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents will be restricted to appropriate individuals. Requests to use specimens stored in the repository must be approved. SOPs ensure that any changes in informed consent made by a patient and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance.

5.5.5 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any tumor tissue blocks or FFPE specimens that are remaining at the completion of the protocol will be stored at the Laboratory of Pathology. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the patient withdraws consent the participants' data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2.**

5.6 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.6.1 Description of The Scope of Genetic/Genomic Analysis

Tumor and blood or saliva specimens will be collected, and genomic analyses will be performed . Tumor genetic material will undergo evaluation for whole exome sequencing and RNA sequencing

evaluating for possible neoantigens and to provide the immunologic microenvironmental landscape respectively. Genetic material containing germline DNA, including blood or saliva will be obtained for whole exome sequencing, which will be performed to combine with WES data from tumor tissue to identify tumor specific somatic mutations and neoantigens.

The genomic tests to determine the mutation burden are going to be performed by CLIA laboratories including Laboratory of Pathology and Genetic Branch. Formalin fixed, and paraffin embedded (FFPE) tumor tissue will be used for genomic material extraction. The whole exome sequencing will be performed to generate the tumor mutation burden from the non-synonumous mutations. Tumor genomic analysis will not be repeated if already performed on another NIH protocol.

5.6.2 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. For the purpose of this study, clinically actionable findings are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists. Subjects will be contacted at that time with a request to provide a blood sample to be sent to a CLIA certified laboratory.

5.6.3 A Certificate of Confidentiality will be obtained for the study as described in section **13.4**

5.6.4 Genetic Counseling

If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling with the NCI Genetics Branch to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense). This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Document AEs from the first study intervention, Day 1 through 100 days after the study intervention was last administered. Beyond 100 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE only if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Grade 1 AEs will not be collected.

All deaths will be recorded.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

Data will be shared through*:*

- An NIH-funded or approved public repository: clinicaltrials.gov, dbGaP.
- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

Data will be shared:

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

For the purposes of this study, patients should be re-evaluated for response every 8 weeks. Response and progression will be evaluated in this study using the iRANO (Immunotherapy Response Assessment in Neuro-oncology) criteria that provides special consideration for the development of pseudo progression, given that the treatment is designed to evoke an immune/inflammatory response. The iRANO criteria provide special considerations for immunotherapy response assessment and represent an integration with established RANO criteria. The iRANO criteria are provided and fully explained in **Appendix D.**

6.3.1 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Measurable Disease: Bi-dimensionally measurable lesions with clearly defined margins by GD-DPTA enhanced MRI scan. The maximal diameter and second perpendicular measurement are at least 10 mm in size.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm), are considered non-measurable disease.

6.3.2 Response Criteria

6.3.2.1 Response Criteria: RANO Criteria with the iRANO update **(Appendix D)**

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

- Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.
- No new lesions.
- All measurable and non-measurable lesions must be assessed using the same techniques as baseline.
- Patients must be on no steroids or on physiologic replacement doses only.
- Stable or improved non-enhancing (T2/FLAIR) lesions
- Stable or improved clinically, for clinical signs and symptoms present at baseline and recorded to be disease related

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

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

- Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks.

In the absence of a confirming scan 4 weeks later, this scan will be considered only stable disease.

- No progression of non-measurable disease.
- No new lesions.
- All measurable and non-measurable lesions must be assessed using the same techniques as baseline.
- The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.
- Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.
- Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

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

- > 25% increase in sum of the products of perpendicular diameters of enhancing lesions (over best response [smallest tumor size] or baseline if no decrease) on stable or increasing doses of corticosteroids
- Any new enhancing measurable lesion that when added to the change in initial tumor(s) exceeds a 25% increase in cross-sectional area.
- Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication side effects, complications of therapy, cerebrovascular events, infection, etc.). The definition of clinical deterioration is left to the discretion of the investigator but it is recommended that a decline in the Karnofsky Performance Score (KPS) from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration, unless attributable to co-morbid events or changes in corticosteroid dose.
- Failure to return for evaluation due to death or deteriorating condition

Classification of progressive disease may be deferred for up to three months for patients with initial radiographic findings consistent with progressive disease (criteria a and b above) as detailed below. However, if follow-up imaging after three months confirms progression or if the patient experiences significant clinical decline at any time, the date of actual progression will be back-dated to the first date that the patient met criteria for progression and such patients should discontinue further immunotherapy.

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

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

Unknown Response Status: Progressive disease has not been documented and one or more measurable or non-measurable lesions have not been assessed.

Patients who require increased corticosteroids within two weeks of MRI assessment (relative to the dose taken at the time of the prior assessment) cannot be classified as CR, PR or SD and should be classified as non-evaluable at that time point. Conversely, patients who decrease corticosteroids within two weeks of MRI assessment (relative to the dose taken at the time of the prior assessment) cannot be classified as PD and should be classified as non-evaluable.

6.3.3 Duration of Response

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

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.4 Progression-free survival

Progression free survival is defined from the day of study entry until imaging is confirmed to show disease progression. Given the anticipated high rate of pseudo progression, patients with worsening lesions but without significant clinical decline may continue on treatment at the PI discretion. Subsequent confirmation of disease progression will mandate that the date of progression be defined as the date imaging worsening was first discovered. Patients who undergo diagnostic biopsy or resection uncovering evidence of pseudo progression will NOT be declared as having disease progression and will be able to resume protocol treatment.

6.3.5 Overall survival

Overall survival: Defined as the time from treatment initiation to the time of death.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm50).

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP Office Of Compliance And Training / IRB Reporting

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI Clinical Director Reporting

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH Required Data and Safety Monitoring Plan

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis weekly when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this

treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death
- A life-threatening adverse event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1.** All serious adverse events recorded from time of the first investigational product administration must be reported to the sponsor**.**

8.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section **8.2**

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 WAIVER OF EXPEDITED REPORTING TO CCR

As death due to disease progression is part of the study objectives (PFS), and captured as an endpoint in this study, they will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to section **8.3.**

8.5 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATOR (BRISTOL MYERS SQUIBB)

8.5.1 Non-Serious Adverse Event Collection and Reporting

The study team will send:

Non-serious 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.

The collection of non-serious AE information should begin at initiation of study drug. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.

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

8.5.2 Serious Adverse Event Collection and Reporting

The CCR Safety will send all reports to the manufacturer as described below:

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur after the first dose of study drug and within 100 days of discontinuation of dosing.

All SAEs must be collected that relate to any protocol-specified procedure. The investigator should report any SAE that occurs after these time periods that is believed to be related to study drug or protocol-specified procedure.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours of Sponsor being aware of the event. SAEs must be recorded on MedWatch form 3500a or equivalent; pregnancies on a Pregnancy Surveillance Form (**Appendix E**).

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 any 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 of Sponsor being aware of the event to the BMS (or designee) using the same procedure used for transmitting the initial SAE report.

The Sponsor will reconcile the clinical database SAE cases (case level only) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms.com). Frequency of reconciliation should be every 3 months and prior to the database lock or final data summary. BMS GPV&E will email, upon request from the Investigator, the GPV&E reconciliation report. Requests for reconciliation should be sent to aepbusinessprocess@bms.com. The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS.

All SAEs should be followed to resolution or stabilization.

8.6 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here:
https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.6.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents. GCP or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING PLAN

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL HYPOTHESIS

10.1.1 Primary Endpoint: PFS at 6 months

Hypothesis: The PFS6 for HMP cohort is 30% or better, and PFS6 for NHMP is 39% or better.

Simon's optimal two-stage design [57] will be used for conducting the trial for the HMP and NHMP cohorts independently. Specifically, for the HMP cohort, the null hypothesis is that the PFS6 is 0.1, and the alternative hypothesis is that the PFS6 is 0.3. The trial is carried out in two stages. In stage I, a total number of 10 patients is accrued. If 9 or more patients progressed by 6 months, the cohort will be terminated early; otherwise, additional 19 patients will be accrued in stage II, resulting in a total sample size of 29. Among these 29 patients, if 6 or more patients are progression-free at 6 months, we reject the null hypothesis and claim that the treatment is promising for patients with HMP IDH-mutant gliomas. The design controls the type I error rate at 0.05 and yields the power of 0.8.

For the NHMP cohort, the null hypothesis is that the PFS6 is 0.186, and the alternative hypothesis is that the PFS6 is 0.386. The trial is carried out in two stages. In stage I, a total number of 15 patients is accrued. If 3 or more patients are progression-free at 6 months, the cohort will be moved to stage II and additional 15 patients will be accrued in stage II, resulting in a total sample size of 30. Among these 30 patients, if 10 or more patients are progression-free at 6 months, we reject the null hypothesis and claim that the treatment is promising for patients with NHMP IDH-mutant gliomas. The design controls the type I error rate at 0.05 and yields the power of 0.8.

Rationale for the target PFS6 in HMP and NHMP cohorts:

Due to the rarity of the disease, there is lack of large scale of studies to provide the accurate survival data of this subset of patients. Based on our anecdotal experience, the HMP IDH-mutant recurrent gliomas have similar course of disease progression as recurrent glioblastoma, while the NHMP ones have similar course as recurrent anaplastic astrocytoma. Wong et al. reported that a progression free survival rate at 6 months (PFS6) is 31% and the median progression free survival is only 13 weeks based on eight Phase II clinical trials that included 150 patients with recurrent anaplastic astrocytoma[58, 59]. In the pivotal clinical trial that led to the accelerated FDA approval for TMZ as the treatment of recurrent AA, the PFS6 and median overall survival were reported as 46% and 13.6 months respectively[59]. Based on these, we set the PFS targets (P0=30% and P1=50%) for NHMP. In the same study, the PFS at 6 for GBM is 15% and median PFS is 9 weeks. Therefore, we used 10% as P0 for HMP gliomas in this proposed study.

10.1.1.1 Sample Size Determination

A maximum of 29 patients with HMP IDH-mutant gliomas, and a maximum of 30 patients with NHMP IDH-mutant gliomas will be enrolled in this phase II trials. Under the design described previously and given the type I error rate of 0.05, we have 80% power to reject the null hypothesis PFS6 = 0.1 for the HMP cohort given the alternative hypothesis PFS6 = 0.3; and 80% power to reject the null hypothesis PFS6 = 0.19 for the NHMP cohort given the alternative hypothesis PFS6 = 0.39.

Up to 59 study patients who undergo at least 1 cycle (2 infusions of nivolumab) is required. As we take into account approximately 20% of screen failures/non-evaluable patients, the overall accrual ceiling is 70 study patients.

10.2 POPULATIONS FOR ANALYSES

10.2.1 Evaluable for toxicity:

All patients will be evaluable for toxicity from the time of their first treatment with nivolumab.

10.2.2 Evaluable for objective response:

Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

10.2.3 Evaluable Non-Target Disease Response:

Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

10.3 STATISTICAL ANALYSES

10.3.1 Analysis of the Primary Endpoints

Point estimate and its standard deviation and 95% confidence interval will be calculated for the PFS6 for HMP cohort and NHMP cohort, respectively. Kaplan-Meier estimates will be used to describe the PFS for each cohort. The log-rank test will be used to compare the PFS of two cohorts.

10.3.2 Analysis of the Secondary Endpoint(s)

The similar analyses will be performed for PFS at 12 months as those for PFS6. We will assess the relationship between the neoantigen burden in tumor prior to the treatment and the PFS using the proportional hazard model. The neoantigen burden and HMP status (HMP/NHMP) will be included in the model as the covariates, and their potential interaction will be assessed. Other important baseline prognostic factors will also be included in the model to adjust for the potential confounders. We will evaluate the relationship between the neoantigen burden in tumor prior to the treatment and the objective response using logistic regression with the neoantigen burden and HMP status as covariates. Linear mixed model will be used to examine the trajectory of the longitudinal measures of symptom burden quantified using the MDASI-Brain tumor module. Random intercept will be included to account for the within-subject correlation and HMP status will be included as a covariate.

10.3.2.1 Patient Reported Outcomes

Received MDASI-BT forms will be checked versus the timing schedule and considered as valid if they fall within ten days of the scheduled assessment window. Compliance rates will be calculated as the number of received valid forms over the number of expected forms. Differences between groups in compliance will be tested by use of Fisher's exact test at every time point.

We will use descriptive statistics to describe how patients rate symptom severity and interference with function at each time point. Error bar graphs for each of the symptoms will be constructed at each time point. The proportion of patients rating their symptoms to be 5 or greater (on a 0-10 scale) will also be reported. We will construct individual patient profiles for each of the selected

symptoms to describe the individual patients' patterns of change over time. We will calculate the mean symptom severity and mean symptom interference at the time of clinical evaluation. Estimates of differences in the mean symptom severity and mean symptom interference between responders and non-responders will be estimated in the intent to treat population. All patients with at least one valid questionnaire will be included in the analyses. Questionnaires completed at study registration will be considered baseline. All questionnaire data received after randomization will be used in the primary analyses.

Differences of at least 2 points will be classified as the minimum clinically meaningful change in the symptom severity and symptom interference measures. For example, an increase of 2 points or more would mean a moderate improvement, whereas a decrease of 2 points or more would be interpreted as moderate worsening. For individual symptoms, a rise in a symptom score means deterioration, whereas a reduced score means improvement of the specific symptom.

10.3.3 Safety Analyses

The type and grade of AE will be summarized using frequency tables.

10.3.4 Planned Interim Analyses

For the HMP cohort, the trial is carried out in two stages. In stage I, a total number of 10 patients is accrued. If 9 or more patients progressed by 6 months, the cohort will be terminated early; otherwise, additional 19 patients will be accrued in stage II, resulting in a total sample size of 29. Among these 29 patients, if 6 or more patients are progression free at 6 months, we reject the null hypothesis and claim that the treatment is promising for patients with HMP IDH-mutant gliomas.

For the NHMP cohort, the trial is carried out in two stages. In stage I, a total number of 15 patients is accrued. If 3 or more patients are progression-free at 6 months, the cohort will be moved to stage II and additional 15 patients will be accrued in stage II, resulting in a total sample size of 30. Among these 30 patients, if 10 or more patients are progression free at 6 months, we reject the null hypothesis and claim that the treatment is promising for patients with NHMP IDH-mutant gliomas.

10.3.5 Exploratory Analyses

Logistic regression will be carried out to investigate if a change in immunocompetence that is defined by peripheral blood mononuclear cell (PBMC) assays and phenotyping over the course of the study treatment correlates with the response and/or treatment-related toxicity. Proportional hazard model will be used to evaluate its relationship with the PFS. Similar exploratory analyses will be performed for evaluate potential correlations between immune infiltrates profiles (and expansion of specific clones) and clinical response.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

The investigational study agent is provided by Bristol-Myers Squibb under a Clinical Trial Agreement (#1097).

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

This study was designed to include women and minorities but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender. No exclusion to this study will be based on race. Minorities will actively be recruited to participate.

12.2 PARTICIPATION OF CHILDREN

Children (younger than 18 years) will not be included in this protocol due to the limited data on nivolumab in children and the different biology of childhood malignancy.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to provide consent are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. For subjects that lose the ability to consent due to tumor progression, they will be taken off treatment but will have the opportunity to remain on study in long term follow-up for collection of outcomes for overall survival. If the reason for incapacity is due to a reason other than definitive disease progression and they are felt to be responding to the treatment under study, they will have the opportunity to continue this therapy if they have a LAR to provide ongoing consent.

All subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see section **12.8** for consent procedure.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS FOR ALL PARTICIPANTS

12.5 BENEFITS

The study drug may help to control the disease. Future patients may benefit from what is learned.

There is no data for this treatment in the recurrent glioma with hypermutated phenotype. The condition has never been studied in the clinical studies. The research is likely to yield generalizable knowledge about the efficacy of nivolumab in this rare condition.

12.6 RISKS

12.6.1 Risks from Study Drug

The primary risk to patients participating in this research study is from the toxicity of nivolumab. Nivolumab is an investigational agent in the treatment of IDH mutated gliomas. The protocol provides for detailed and careful monitoring of all patients to assess for toxicity. Toxicity data will be collected and reviewed to ensure that there were no severe toxicities that would preclude further

patient enrollment. Patients will be treated with therapeutic intent and response to the therapy will be closely monitored.

12.6.2 Blood Collection

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop.

12.6.3 Imaging

Subjects receive brain MRIs. People are at risk for injury from the MRI magnet if they have some kinds of metal in their body. It may be unsafe for subjects to have an MRI scan 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), metal prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, tattoos, an implanted delivery pump, or shrapnel fragments. Welders and metal workers may have small metal fragments in the eye. Subjects will be screened for these conditions before having any MRI scan. In addition, all magnetic objects (like watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room.

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.

Scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling. Participants undergoing gadolinium enhanced MRIs may also be at risk for kidney damage.

12.6.4 Electrocardiogram

Some skin irritation can occur where the ECG/EKG electrodes are placed. The test is completely painless, and generally takes less than a minute to perform.

12.6.5 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.6.6 Questionnaires risks

Questionnaires may contain questions that are sensitive in nature.

12.6.7 Non-Physical Risks of Genetic Research

12.6.7.1 Risk of receiving unwanted information

Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA sequencing or disease tendencies, or misattributed paternity. Patients will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers.

12.6.7.2 Risk related to possibility that information may be released

This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document.

12.7 RISKS/BENEFITS ANALYSIS FOR ALL PARTICIPANTS

This study is for patients with glioma. Currently, no successful standard therapy has been established, and the impact of chemotherapy at recurrence has been limited. Participants may obtain better responses with nivolumab.

The primary risk to patients participating in this research study is from the toxicity of nivolumab, that will be closely monitored.

Furthermore, we believe that genetic analyses planned for both normal and malignant tissues will pose no more than a minimal risk to the patients or their family members, as the results will not be reported with any identifiable information. The codes linking the subject with samples will be maintained by the principal investigator and research coordinator in a locked electronic data base, with access only to the PI and research coordinators. The testing will be considered exploratory and investigational, and without clinical utility. Results of these tests will not be shared with participants. There will be data monitoring plan to ensure the safety of the subjects.

12.8 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator* will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

<u>Manual (non-electronic) signature on electronic document</u>:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location, but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

* Please note that consent for treatment must be obtained by a designated appropriately licensed study investigator (e.g., MD, NP, PA, DO). However, study investigators not falling into this category (e.g. RNs) who are designated as able to obtain consent, may do so for non-treatment procedures such as screening.

12.8.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in Section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.8**.

12.8.2 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section 2.2.1 may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NCI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

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

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived by the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 NIVOLUMAB (IND #133973)

14.1.1 Source:

Bristol Myers Squibb will supply investigational nivolumab. Only 100mg, 10mg/ml Nivolumab vials will be provided for this study. There are 5 vials per carton.

14.1.2 Toxicity:

Nivolumab is most commonly associated with immune-related adverse reactions. Most of these, including severe reactions, resolved following initiation of appropriate medical therapy or withdrawal of nivolumab.

Refer to investigator brochure for detailed toxicity information.

14.1.3 Formulation and preparation

Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate, sodium chloride, mannitol, diethylenetriamine pentacetic acid (pentetic acid) and polysorbate 80 (Tween® 80), pH 6.0.

Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to concentrations no less than 1 mg/mL.

14.1.4 Stability and Storage

Store intact vials at 2°C to 8°C (36°F to 46°F); do not freeze. Protect from light. Do not shake. Shelf-life surveillance of the intact vials is ongoing.

Store the prepared infusion solution at room temperature or refrigerated. Do not freeze solutions prepared for infusion. Refer to investigator brochure for detailed stability information.

14.1.5 Administration procedures

Intravenous infusion 30 to 60 minutes. Do not administer as an IV push or bolus injection.

Administer through a 0.2 micron to 1.2-micron pore size, low-protein binding in-line filter.

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16 APPENDICES

16.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

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

16.2 APPENDIX B: NEUROLOGIC EVALUATION

0= No neurologic symptoms, fully active at home/work without assistance

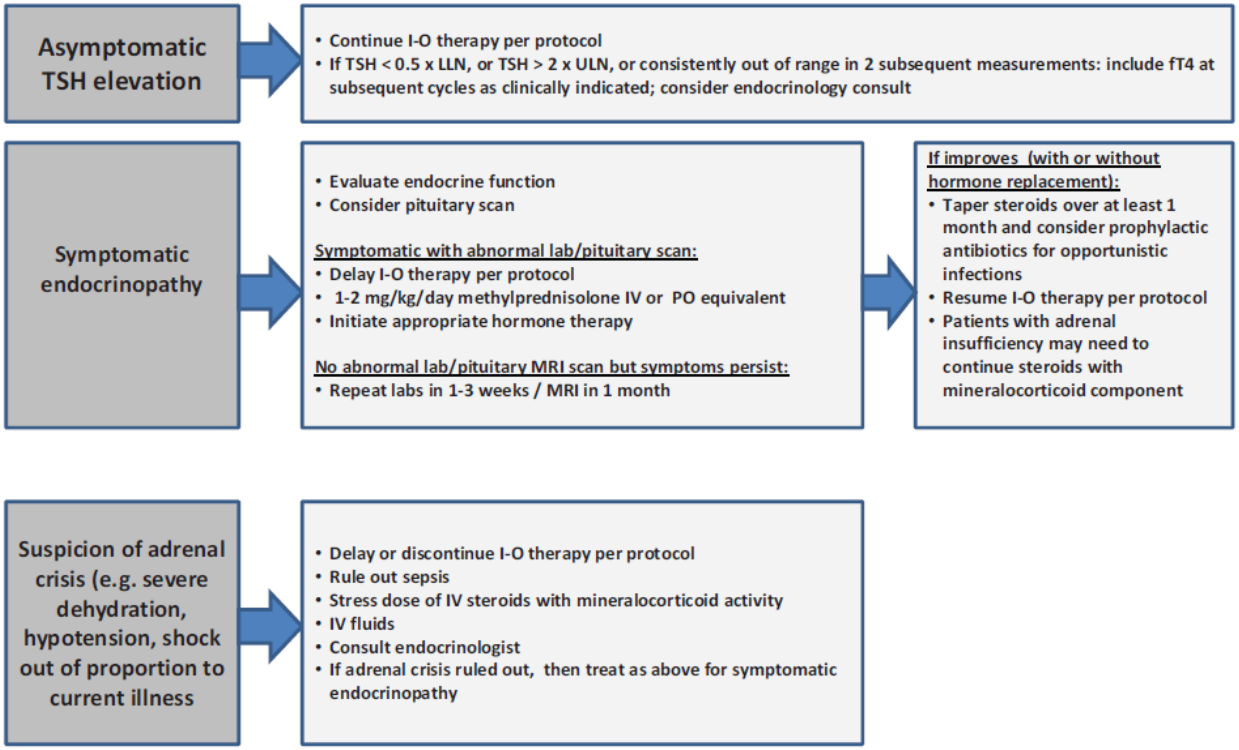
1= Minor neurologic symptoms, fully active at home/work without assistance
2= Moderate neurologic symptoms, fully active at home/work but requires assistance
3= Moderate neurologic symptoms, less than fully active at home/work and requires assistance

4= Severe neurologic symptoms, totally inactive requiring complete assistance at home or institution, unable to work

16.3 APPENDIX C: MANAGEMENT ALGORITHMS FOR ENDOCRINOPATHY, GASTROINTESTINAL, HEPATIC, NEUROLOGICAL, PULMONARY, RENAL, AND SKIN ADVERSE EVENTS

Endocrinopathy Management Algorithm

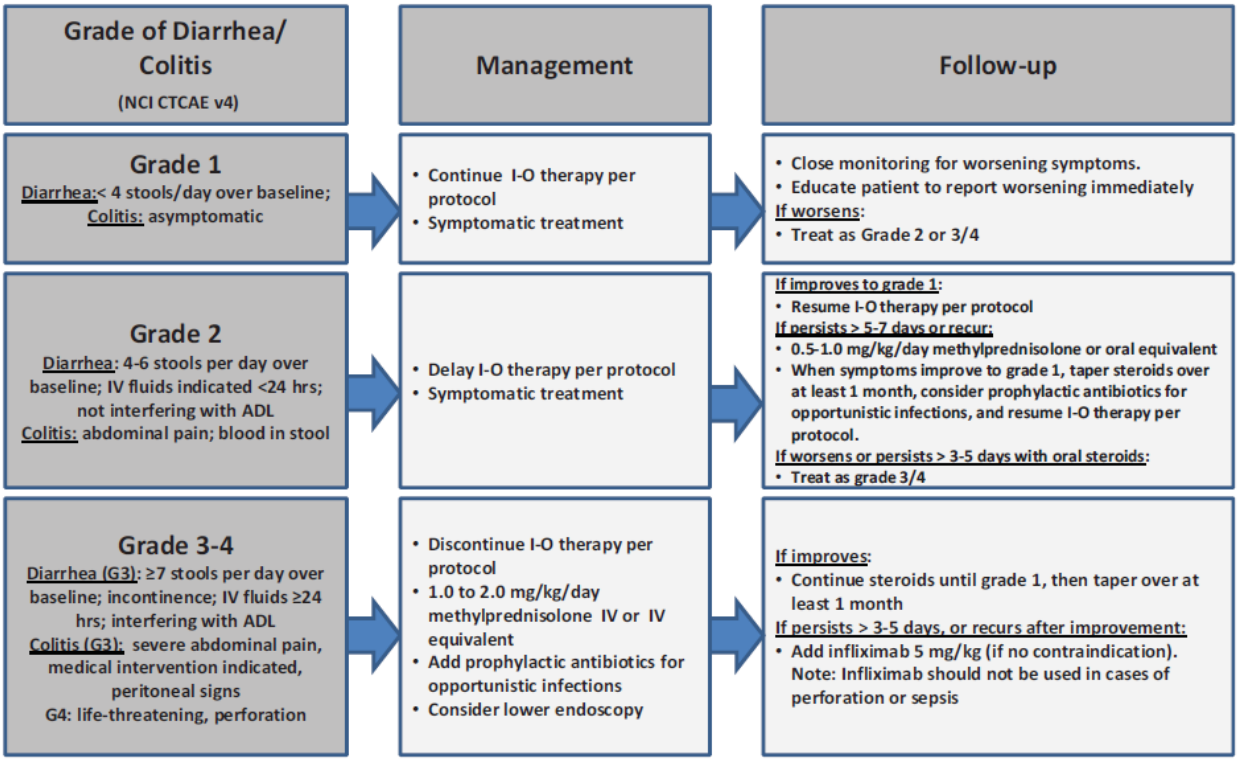
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

GI Adverse Event Management Algorithm

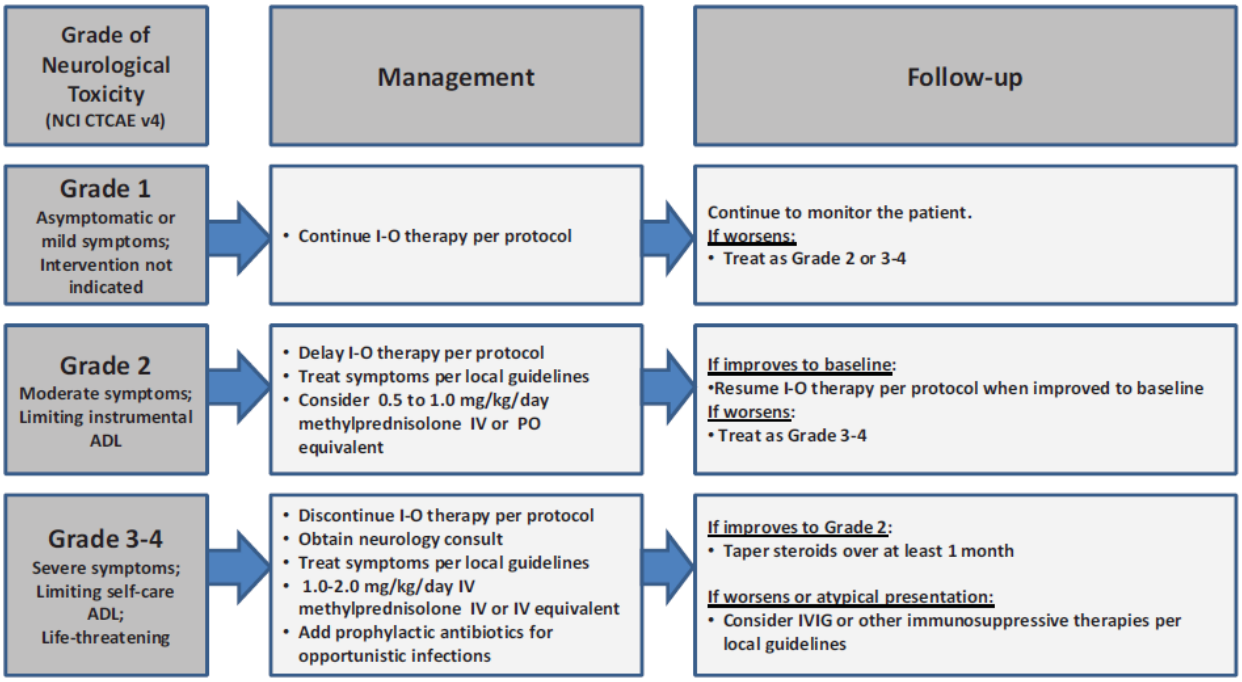
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



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

Neurological Adverse Event Management Algorithm

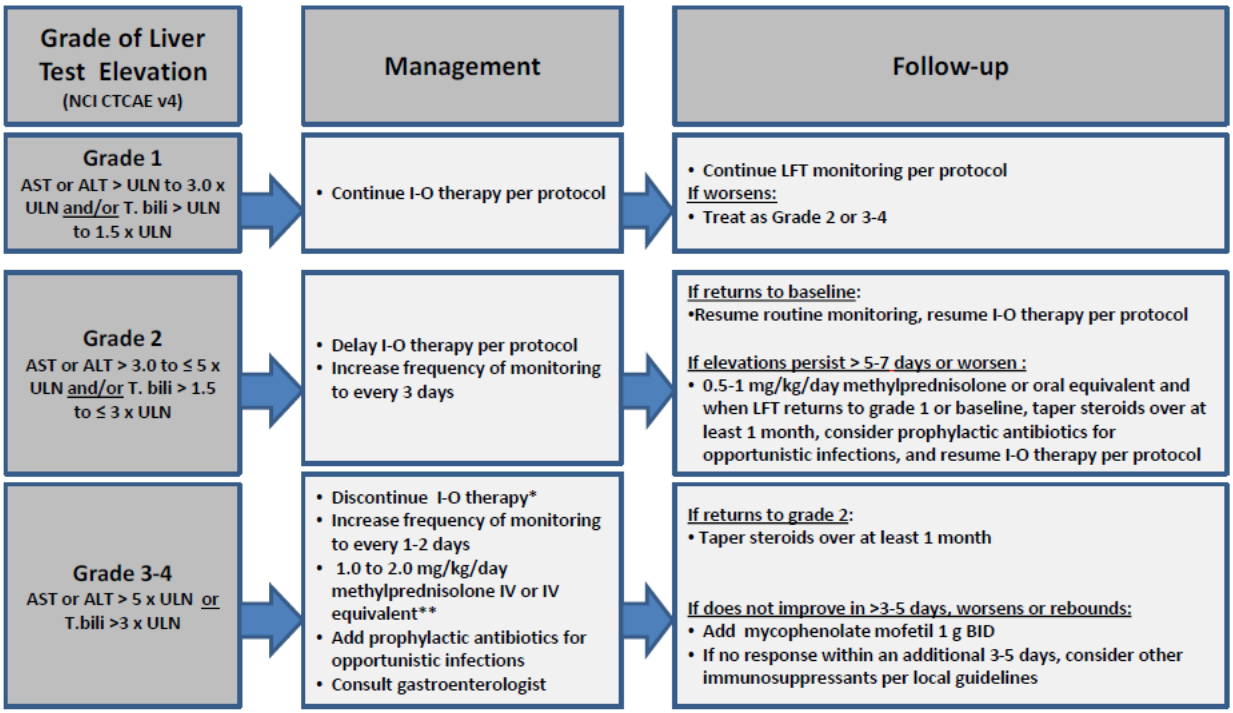
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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

Hepatic Adverse Event Management Algorithm

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



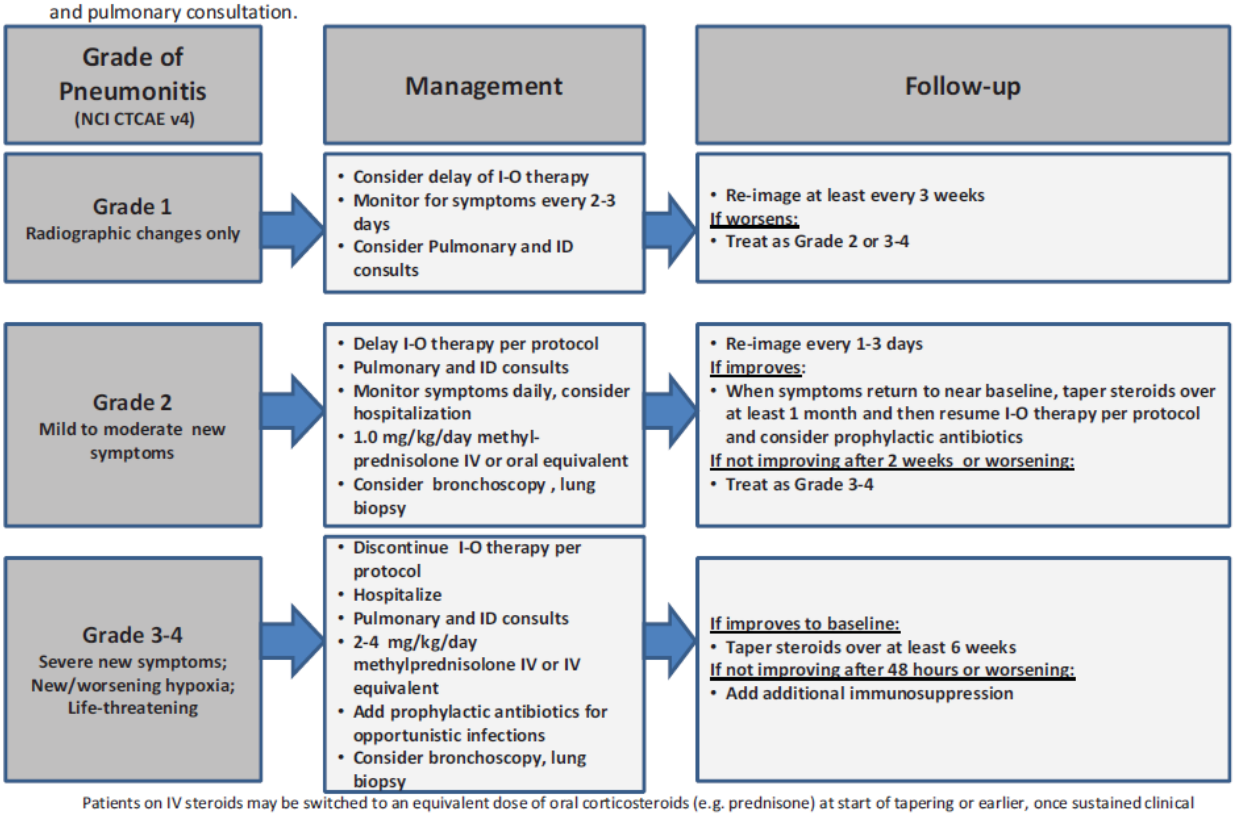
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

***I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN or T.bili ≤ 5 x ULN.**

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Pulmonary Adverse Event Management Algorithm

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



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

Renal Adverse Event Management Algorithm

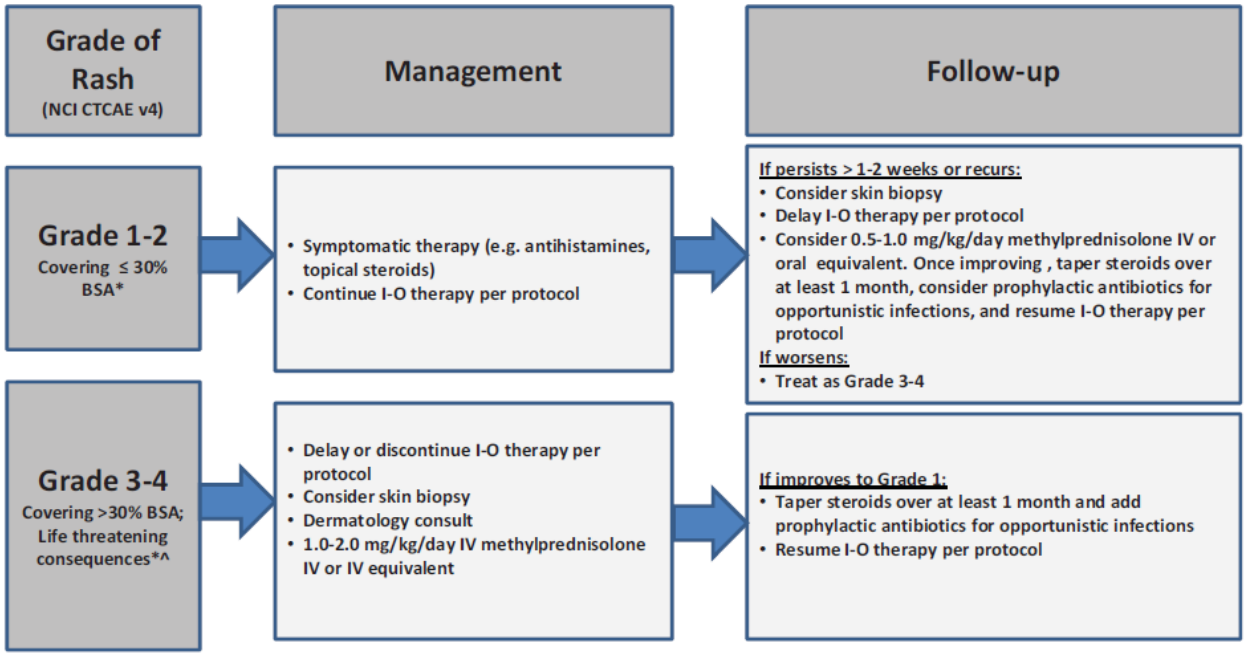
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy

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

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

Skin Adverse Event Management Algorithm

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



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

16.4 APPENDIX D: RANO CRITERIA AND iRANO TREATMENT ALGORITHM

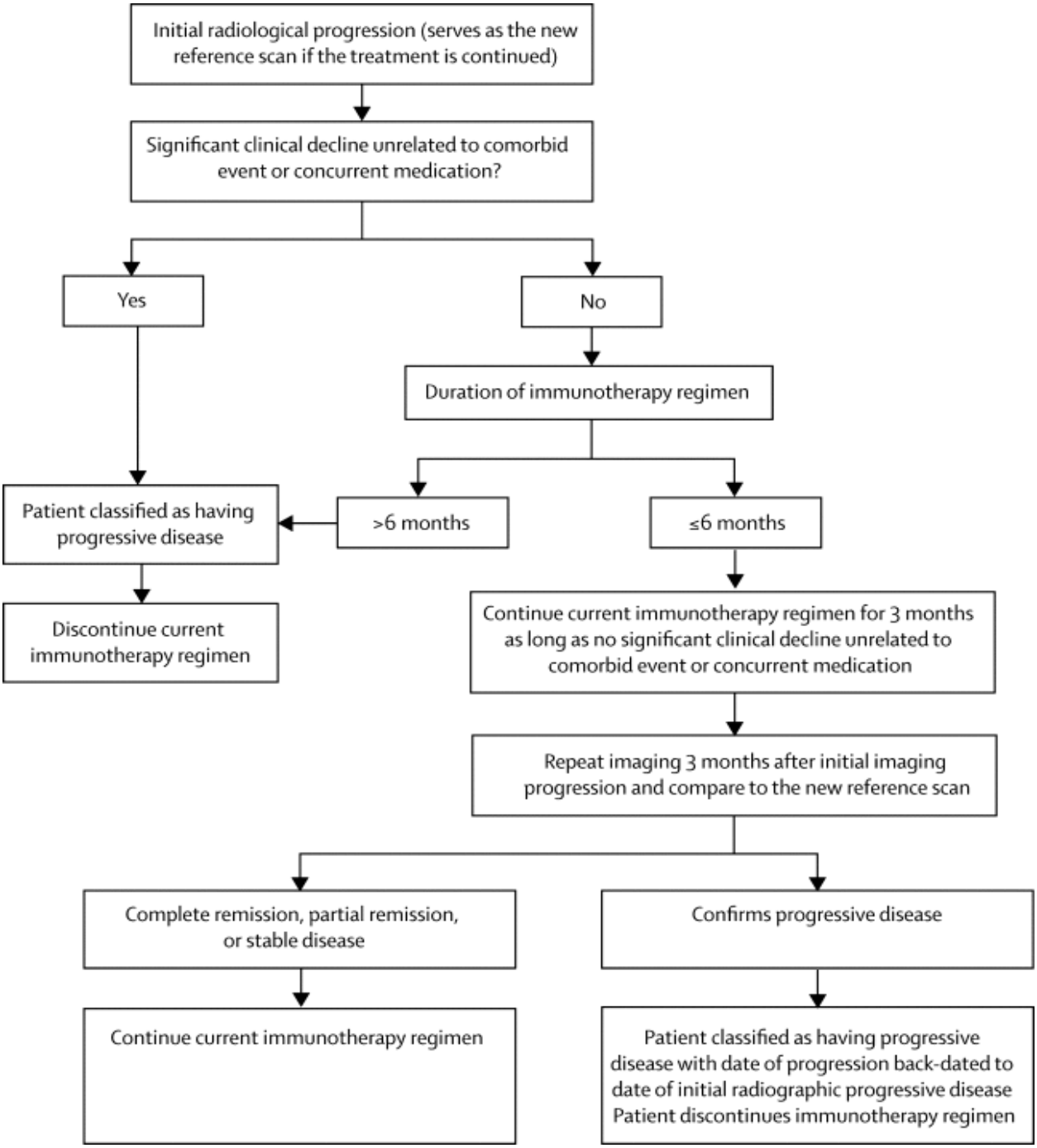
Modified RANO Response Criteria

	CR	PR	SD	PD#
T1-Gd +	None	≥50% decrease	<50% decrease- <25% increase	≥25% increase including new lesion(s)*
T2/FLAIR	Stable or decrease	Stable or decrease	NA	NA
New Lesion	None	None	None	Present (contributing to lesion size)*
Corticosteroids	None	Stable or decrease	Stable or decrease	Stable or increasing
Clinical Status	Stable or increase	Stable or increase	Stable or increase	Decrease*
Requirement for Response	All	All	All	Any*

CR=complete response; **PR**=partial response; **SD**=stable disease; **PD**=progressive disease; **NA**= not applicable

#: Progression occurs when any of the criteria with * is present; radiologic confirmation of progression is permitted as described below

Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration


Initial radiological progression (serves as the new reference scan if the treatment is continued)
Significant clinical decline unrelated to comorbid event or concurrent medication?
Yes
No
Duration of immunotherapy regimen
Patient classified as having progressive disease
>6 months
≤6 months
Discontinue current immunotherapy regimen
Continue current immunotherapy regimen for 3 months as long as no significant clinical decline unrelated to comorbid event or concurrent medication
Repeat imaging 3 months after initial imaging progression and compare to the new reference scan
Complete remission, partial remission, or stable disease
Confirms progressive disease
Continue current immunotherapy regimen
Patient classified as having progressive disease with date of progression back-dated to date of initial radiographic progressive disease
Patient discontinues immunotherapy regimen

16.5 APPENDIX E: PREGNANCY FORM

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part I
(Antepartum Information)

PATIENT IDENTIFIER:	CARES NUMBER: (BMS ONLY)	LOCAL COUNTRY NUMBER: (BMS ONLY)

BMS RECEIPT DATE (BMS USE ONLY) D D M M M Y Y

GPV&E RECEIPT DATE (BMS USE ONLY) D D M M M Y Y

REPORT TYPE: ☐ SPONTANEOUS OR ☐ STUDY ☐ INITIAL REPORT OR ☐ FOLLOW-UP REPORT

COUNTRY

EVENT: PREGNANCY ☐ MATERNAL DRUG EXPOSURE OR ☐ PATERNAL DRUG EXPOSURE ☐ PROSPECTIVE REPORT OR ☐ RETROSPECTIVE REPORT

WERE THERE ANY ADDITIONAL MATERNAL/PATERNAL ADVERSE EVENTS ? ☐ NO ☐ YES
IF YES, REPORT THE ADVERSE EVENTS APPROPRIATELY (FOR STUDIES, REFER TO STUDY-SPECIFIC INSTRUCTIONS)

MATERNAL INFORMATION DATE OF BIRTH: D D M M M Y Y Y Y

AGE AT CONCEPTION:

HEIGHT: ☐ inches ☐ cm

WEIGHT: ☐ lb ☐ kg

RACE: ☐ WHITE ☐ BLACK ☐ ASIAN ☐ AMERICAN INDIAN OR ALASKAN NATIVE ☐ NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER ☐ OTHER RACE:

NUMBER OF PREGNANCIES INCLUDING THIS ONE

NUMBER OF BIRTHS

NUMBER OF LIVING CHILDREN

ONSET DATE LAST MENSTRUAL PERIOD (LMP): D D M M M Y Y

APPROXIMATE DATE OF CONCEPTION: D D M M M Y Y

ESTIMATED DATE OF DELIVERY: D D M M M Y Y

DATE PREGNANCY WAS CONFIRMED: D D M M M Y Y

TEST METHOD: ☐ SERUM ☐ URINE

ESTIMATED GESTATIONAL AGE WHEN PREGNANCY DIAGNOSED: WEEKS

DETERMINED BY: ☐ FETAL ULTRASOUND ☐ DATE FROM LMP

CONTRACEPTION AT TIME OF CONCEPTION: ☐ NO ☐ YES ☐ UNKNOWN (IF YES, SPECIFY)

RELEVANT MATERNAL MEDICAL HISTORY/RISK FACTORS	DATE OF ONSET D D M M M Y Y	IF APPLICABLE SPECIFY PERTINENT DETAILS

PATERNAL INFORMATION: AGE YEARS DATE OF BIRTH: D D M M M Y Y Y Y

RELEVANT PATERNAL MEDICAL HISTORY/RISK FACTORS	DATE OF ONSET D D M M M Y Y	IF APPLICABLE SPECIFY PERTINENT DETAILS

28 March 2011 CT SOP 108 FRM 01.v03 Page ___ of ___

Alterations to this form are not permitted unless otherwise specified in the governing Procedural Document.

Original - Bristol-Myers Squibb; Copy - Retained by Investigator

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part I (Antepartum Information)

Patient Identifier:	CARES Number: (BMS only)	Local Country Number: (BMS only)

Medication name and indication	Pregnancy Related to Medication?*	Dose and units	Freq	Route**	Period(s) of drug exposure***	Oncology drugs only	Start and Stop dates (D D M M M Y Y)
1. Indication ☐ Maternal or ☐ Paternal ☐ Non-study or ☐ Study	☐ Not related ☐ Related					Cycle #: Cumulative dose with units	or ☐ ongoing
2. Indication ☐ Maternal or ☐ Paternal ☐ Non-study or ☐ Study	☐ Not related ☐ Related					Cycle #: Cumulative dose with units	or ☐ ongoing
3. Indication ☐ Maternal or ☐ Paternal ☐ Non-study or ☐ Study	☐ Not related ☐ Related					Cycle #: Cumulative dose with units	or ☐ ongoing
4. Indication ☐ Maternal or ☐ Paternal ☐ Non-study or ☐ Study	☐ Not related ☐ Related					Cycle #: Cumulative dose with units	or ☐ ongoing
5. Indication ☐ Maternal or ☐ Paternal ☐ Non-study or ☐ Study	☐ Not related ☐ Related					Cycle #: Cumulative dose with units	or ☐ ongoing
6. Indication ☐ Maternal or ☐ Paternal ☐ Non-study or ☐ Study	☐ Not related ☐ Related					Cycle #: Cumulative dose with units	or ☐ ongoing
7. Indication ☐ Maternal or ☐ Paternal ☐ Non-study or ☐ Study	☐ Not related ☐ Related					Cycle #: Cumulative dose with units	or ☐ ongoing

* Mandatory for all studies

**Route:
1 = Oral 2 = Intravenous 3 = Subcutaneous 4 = Other

***Period(s) of drug exposure: (include all that apply)
0 = Prior to conception 1 = 1ST trimester 2 = 2nd trimester
3 = 3RD trimester 4 = Labor & delivery 5 = Unknown

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part I
(Antepartum Information)

PATIENT IDENTIFIER:	CARES NUMBER: (BMS ONLY)	LOCAL COUNTRY NUMBER: (BMS ONLY)

PRENATAL DIAGNOSTIC TESTING	BASE-LINE	DATE D D M M M Y Y	TEST RESULTS UNITS	NORMAL RANGE LOW	NORMAL RANGE HIGH
	☐				
	☐				
	☐				
	☐				
	☐				
	☐				
	☐				

DESCRIBE RESULTS IN DETAIL, IF APPLICABLE:

REPORTER INFORMATION: ☐ BMS STUDY INVESTIGATOR ☐ NON-BMS STUDY SPONSOR ☐ OTHER*

*QUALIFICATION: (COMPLETE ONLY IF "OTHER" IS CHECKED)

☐ PHYSICIAN ☐ PHARMACIST ☐ NURSE/NURSE PRACTITIONER ☐ OTHER HEALTH PROFESSIONAL
☐ CONSUMER ☐ ATTORNEY ☐ OTHER NON-HEALTH PROFESSIONAL

PERSON COMPLETING THE FORM (IF DIFFERENT FROM INVESTIGATOR/SPONSOR) :

PRINTED NAME

SIGNATURE

DATE: D D M M M Y Y

INSTITUTION/ORGANIZATION:

STREET ADDRESS: | CITY: | STATE/PROVINCE:

POST CODE: | COUNTRY: | PHONE NUMBER:

INVESTIGATOR/SPONSOR/OTHER:

LAST NAME

FIRST NAME | MIDDLE INITIAL

SIGNATURE:

DATE: D D M M M Y Y

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part II (Pregnancy Outcome)

Patient Identifier:	CARES Number: (BMS only)	Local Country Number: (BMS only)

Pregnancy outcome:

Mode of delivery : ____ Labor/delivery complications ☐ No ☐ Yes*
If yes, specify ____

☐ Single gestation ☐ Multiple gestation (# ☐ of ☐)
complete an outcome form for each fetus/infant

Date pregnancy ended: D D M M M Y Y
Gestational age at outcome ____ weeks ☐ Unknown
Assessed by: ☐ Obstetrical dates ☐ Fetus/infant physical exam

Did obstetrical complications or maternal/paternal medical conditions occur during this pregnancy ?
☐ No ☐ Yes*
☐ Unknown If yes, specify: ____

*For any complications noted above, report the adverse event appropriately (For studies, refer to study-specific instructions)

Gender:	Birth weight:	Birth length:	Head circumference:	APGAR score:
☐ Male ☐ Female ☐ Unknown	☐☐/☐☐ lbs/oz ☐☐☐☐ grams	____ ☐ inches ☐ cm	____ ☐ inches ☐ cm	1 Min. ____ 5 Min. ____

☐ Live birth normal (Proceed to part III)

☐ Live birth abnormal ☐ Fetal death ☐ Neonatal death (If any are checked, complete sections below)

☐ Pre-term ☐ Term ☐ Post term
☐ Small for gestational age
☐ Intrauterine growth retardation
☐ Drug withdrawal syndrome in the neonate
☐ Malformation (specify below)
☐ Post-natal/neonatal complications (e.g. perinatal asphyxia, infection, respiratory distress) (specify): ____

Fetal death
☐ Ectopic ☐ Miscarriage/spontaneous abortion ☐ Stillbirth
☐ Induced abortion/elective termination
Autopsy/pathology report ☐ No ☐ Yes ☐ Unknown

Neonatal death:
Cause : ____ Date: D D M M M Y Y

Placental abnormalities ☐ No ☐ Yes ☐ Unknown
If yes, specify: ____
Pathology report available ☐ No ☐ Yes ☐ Unknown

Family history of congenital abnormalities/birth defects:
☐ No ☐ Yes ☐ Unknown
If yes, specify: ____

Prior pregnancies with congenital abnormalities/birth defects: ☐ No ☐ Yes
If yes, specify #/type : ____

Prior stillbirths: ☐ No ☐ Yes
If yes, specify # : ____

Prior spontaneous abortions: ☐ No ☐ Yes
If yes, specify #: ____

Specify any prior pregnancy complications: ____

History of fertility treatments (e.g. IVF):
☐ No ☐ Yes If yes, specify: ____

Describe any congenital malformations/abnormalities, structural defects and other fetal/neonatal complications:

Causality (mandatory for studies)

In the investigator's opinion, was the defect/medical problem related to medication under study ? : ☐ Not related ☐ Related
If related, please comment on specific event(s) and medication(s) below:
If not related, indicate what the defect/medical problem was attributed to:

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part III (Infant Follow-up)

PATIENT IDENTIFIER:	CARES NUMBER: (BMS ONLY)	LOCAL COUNTRY NUMBER: (BMS ONLY)

CURRENT INFANT AGE: ☐ AGE UNITS: ☐ DAYS ☐ WEEKS ☐ MONTHS

☐ NO PROBLEMS ☐ MEDICAL PROBLEMS NOTED (SPECIFY AND DESCRIBE FINDINGS AND/OR PLANNED EVALUATIONS; E.G. DIAGNOSTIC TESTING, CONSULTATIONS, ETC)

CAUSALITY (MANDATORY FOR ALL STUDIES): IN THE INVESTIGATOR'S OPINION WERE ANY PROBLEMS NOTED ABOVE RELATED TO THE MEDICATION UNDER STUDY ? ☐ NOT RELATED ☐ RELATED (PLEASE SPECIFY):

MATERNAL BREASTFEEDING: ☐ NO ☐ YES HOW LONG:

MATERNAL DRUGS TAKEN WHILE BREASTFEEDING: ☐ NO ☐ YES (IF YES, SPECIFY)

REPORTER INFORMATION: ☐ BMS STUDY INVESTIGATOR ☐ NON-BMS STUDY SPONSOR ☐ OTHER*

*QUALIFICATION: (COMPLETE ONLY IF "OTHER" IS CHECKED)

☐ PHYSICIAN ☐ PHARMACIST ☐ NURSE/NURSE PRACTITIONER ☐ OTHER HEALTH PROFESSIONAL
☐ CONSUMER ☐ ATTORNEY ☐ OTHER NON-HEALTH PROFESSIONAL

PERSON COMPLETING THE FORM (IF DIFFERENT FROM INVESTIGATOR/SPONSOR) :
PRINTED NAME
SIGNATURE
DATE: D D M M M Y Y

INSTITUTION/ORGANIZATION:

STREET ADDRESS: CITY: STATE/PROVINCE:

POST CODE: COUNTRY: PHONE NUMBER:

INVESTIGATOR/SPONSOR/OTHER:
LAST NAME
FIRST NAME MIDDLE INITIAL

SIGNATURE: DATE: D D M M M Y Y

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Alterations to this form are not permitted unless otherwise specified in the governing Procedural Document.

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