Abbreviated Title: Nivo Recurrent Rare CNS Cancer
NIH Protocol #: 17C0102
Bristol-Myers Squibb Protocol Number: CA209-843

Title: Phase II Trial of the Immune Checkpoint Inhibitor Nivolumab in Patients with Recurrent Select Rare CNS Cancers

Version Date: 09/02/2026

NCT Number: NCT03173950

Coordinating Center: NCI-CONNECT Coordinating Center
Center for Cancer Research, NCI

Principal Investigator: Kevin A. Camphausen, MD,
Neuro Oncology (NOB),CCR,NCI
10 Center Dr. Room B2-3675
Bethesda, MD 20892
Tel: 240-760-6205
Email: camphauk@mail.nih.gov

Drug Name:	Nivolumab
IND Number:	133973
Sponsor:	NCI CCR
Manufacturer:	Bristol-Myers Squibb
Supplier:	Bristol-Myers Squibb

Commercial Agents: None

A DISCLAIMER STATEMENT FOR NCI-CONNECT PROTOCOLS

This is a research protocol of the NCI-CONNECT describing an experimental treatment procedure. It is a privileged document and is not intended to be circulated or used for other purposes. The NCI-CONNECT Consortium assumes no responsibility for its use outside of the constraints of this research protocol or by investigators other than those approved by the Consortium.

PRÉCIS

Background:

- There are more than 130 identified primary tumors of the central nervous system (CNS). Most have an annual incidence of less than 1000 in the United States.
- Given the rarity of each of the tumors listed above, there is a paucity of proven therapies. Most of these neoplasms are treated with maximum surgical resection followed by treatment with external beam radiotherapy. With few exceptions (medulloblastoma, adult ependymoma), there are no effective systemic regimens and even in chemotherapy sensitive disease, most patients with recurrence eventually have no remaining salvage treatments available.
- In the setting of this unmet need, we propose to create a basket protocol that will evaluate the efficacy of the PD-1 inhibitor, nivolumab, in patients with refractory rare central nervous system neoplasms.
- This study seeks to establish effective therapies at recurrence in patients with rare CNS tumors. We hypothesize that this therapy will improve progression free survival and/or objective responses.
- It will be important to determine whether any determined survival benefit is associated with improvements in symptoms or does a worsening of symptoms offset the increase in survival. Precedence exists for measuring "non-therapeutic" endpoints in oncology research, and specifically in studies evaluating therapeutic benefit in patients with CNS tumors. 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 (MDASI-BT) and Spine Tumor Module (MDASI-SP) allow for the self-reporting of symptom severity and interference with daily activities for patients with either brain or spinal cord tumors. The availability of validated instruments provides an opportunity to prospectively assess the impact of treatment, both positive and negative, on patients.

Objective:

- Determine the efficacy of nivolumab in a variety of recurrent, refractory primary central nervous system tumors as measured by disease control rate (confirmed CR/PR or durable SD for at least 6 months).

Eligibility:

- Documented recurrent or progressive disease that corresponds to one of the tumors eligible for testing.
- Age ≥ 18 years of age.
- Karnofsky Performance ≥ 70%.
- Tumor tissue available for central review to confirm morphologic diagnosis.
- Tumor tissue or slides must be available for central molecular and immune profiling.

Design:

- This is an open label phase II clinical trial. Patients will be treated with the immune checkpoint inhibitor, nivolumab, at a standard dose of 240 mg intravenously every 2 weeks (+/- 3 days) for cycles 1 through 2, then doses of 480 mg every 4 weeks (+/- 3 days) for a total of 14 additional doses (cycles). A maximum of 18 treatments will be given (64 weeks).
- A cycle will be defined as 4 weeks and patients will undergo efficacy assessments using MR imaging (and/or other imaging tests if applicable) every 2 cycles. Toxicity assessments will occur before the initiation of each cycle and patient outcomes measures (PROs) will be completed at the time of each imaging study (every 2 cycles) but prior to the patient being informed of the imaging results.
- After completion of the planned treatment course or if treatment was stopped because of toxicity, patients will undergo imaging evaluations and PRO measurements every 8 weeks (or 2 months) for one year, then every 3 months for the next year, then every 4 months for the next year and then every 6 months while the patient remains on the protocol. Patients off treatment because of disease progression will not undergo future imaging or PRO assessments on this protocol.
- Bayesian Optimal Phase 2 design (BOP2), will be used to conduct this phase II trial in patients with a variety of recurrent, refractory primary central nervous system tumors.
- The study will be comprised of 2 disease cohorts: heavily pretreated (defined as having received 3 or more prior therapies) and non-heavily pretreated (defined as having received up to 2 prior therapies). Each cohort will be evaluated independently for efficacy.

TABLE OF CONTENTS

PRÉCIS 2
1 INTRODUCTION 8
1.1 Study Objectives 8
1.2 Background and Rationale 9
2 ELIGIBILITY ASSESSMENT AND ENROLLMENT 22
2.1 Eligibility Criteria 22
2.2 Recruitment Strategies 25
2.3 Screening Evaluation 25
2.4 Participant Registration and Status Update Procedures 27
2.5 Baseline Evaluations 29
3 STUDY IMPLEMENTATION 29
3.1 Study Design 29
3.2 Drug Administration 30
3.3 Dose Modifications 30
3.4 Questionnaires (Appendix 16.1) 36
3.5 Telehealth Visits 36
3.6 Study Calendar 39
3.7 Cost and Compensation 43
3.8 Criteria for Removal from Protocol Therapy and Off Study Criteria 43
4 CONCOMITANT MEDICATIONS/MEASURES 45
4.1 Permitted Supportive/Ancillary Care and Concomitant Medications 45
4.2 Prohibited Therapies 46
5 CORRELATIVE STUDIES FOR RESEARCH 46
5.1 Biospecimen Collection (See 16.6) 46
5.2 Sample Storage, Tracking and Disposition 49
5.3 Multi-site instructions for Tissue Collection and Shipment 50
5.4 Samples for Genetic/Genomic Analysis 51
6 DATA COLLECTION AND EVALUATION 52
6.1 Data Collection 52
6.2 Human Data Sharing, including Genomic Data Sharing, and Publication 56
6.3 Response Criteria 57
6.4 Toxicity Criteria 60
7 NIH REPORTING REQUIREMENTS/DATA SAFETY MONITORING PLAN (NIH SITE ONLY) 60

7.1 Definitions....60
7.2 OHSRP Office of Compliance and Training/IRB Reporting....61
7.3 NCI Clinical Director Reporting....61
7.4 NCI Guidance For Expedited Reporting For Multi-Center Trials....61
7.5 NIH Required Data and Safety Monitoring Plan....61
8 SPONSOR PROTOCOL/SAFETY REPORTING....62
8.1 Definitions....62
8.2 Assessment of Safety Events....63
8.3 Reporting of Serious Adverse Events....63
8.4 Safety Reporting Criteria to the Pharmaceutical Collaborator (BMS)....64
8.5 Reporting Pregnancy....65
8.6 Regulatory Reporting for Studies Conducted Under CCR-Sponsored IND....65
8.7 Sponsor Protocol Deviation Reporting....65
9 CLINICAL MONITORING....66
10 STATISTICAL CONSIDERATIONS....67
10.1 Statistical design....67
10.2 Statistical Analysis Plan....69
11 COLLABORATIVE AGREEMENTS....70
11.1 Agreement Type....70
11.2 MULTI-INSTITUTIONAL GUIDELINES....70
12 HUMAN SUBJECTS PROTECTIONS....70
12.1 Rationale For Participant Selection....70
12.2 Participation of Children....71
12.3 Participation of Adults Unable to Give Consent (NCI NOB Only)....71
12.4 Participation of subjects unable to give consent (NCI-CONNECT participating sites)....71
12.5 Evaluation of Benefits and Risks/Discomforts....71
12.6 Risk/Benefit Assessment....72
12.7 Consent Process and Documentation....73
13 REGULATORY AND OPERATIONAL CONSIDERATIONS....74
13.1 Study Discontinuation and Closure....74
13.2 Quality Assurance and Quality Control....75
13.3 Conflict of Interest Policy (NCI Site only)....75
13.4 Confidentiality and Privacy....75
14 PHARMACEUTICAL INFORMATION....76
14.1 Nivolumab (IND# 133973)....76

15 REFERENCES 81

16 APPENDICES 87

16.1 Questionnaires (MDASI-BT & MDASI-SP) 87

16.2 Performance Status Criteria 87

16.3 Neurologic evaluation 88

16.4 Management Algorithms For Endocrinopathy, Gastrointestinal, Hepatic, Neurological, Pulmonary, Renal, And Skin Adverse Events 89

16.5 iRECIST 98

16.6 Flow cytometry techniques for analysis of tumor and peripheral blood 99

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for 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

Determine the efficacy of nivolumab in a variety of recurrent, refractory primary central nervous system tumors [Ependymoma, Medulloblastoma, Parenchymal Pineal Region Tumors (Pineoblastoma, Pineocytoma, Pineal Tumor of Intermediate Differentiation, Papillary Tumor of the Pineal Region), Choroid Plexus Tumors (Carcinoma, Papilloma, Atypical Papilloma), Histone Mutated Gliomas, Gliomatosis Cerebri, ATRT, Malignant/Atypical Meningioma, Gliosarcoma or Primary CNS Sarcoma, Pleomorphic Xanthoastrocytoma (PXA) and Anaplastic Pleomorphic Xanthoastrocytoma (APXA), and tumors formerly known as Primitive Neuro-Ectodermal Tumors (Embryonal Tumor with Multilayered Rosettes, Medulloepithelioma, CNS Neuroblastoma, CNS Ganglioneuroblastoma, CNS Embryonal Tumor NOS); and tumor entities emerging from methylation profiling of CNS-PNETs: CNS neuroblastoma with FOXR2 activation, CNS Ewing sarcoma family tumor with CIC alteration, CNS high-grade neuroepithelial tumor with MN1 alterations, and CNS high-grade neuroepithelial tumor with BCOR alteration] as measured by disease control rate (confirmed CR/PR or durable SD for at least 6 months).

1.1.2 Secondary Clinical Objectives

1.1.2.1 Determine overall survival after initiation of nivolumab for the recurrent or refractory primary CNS tumors listed above measured from study entry.

1.1.2.2 Determine the treatment-related toxicities, particularly immune-related adverse events, associated with this therapeutic regimen.

1.1.2.3 Measure treatment impact and disease response on symptom burden by longitudinal assessments using the MDASI-BT or MDASI-SP questionnaires, including:

- To longitudinally evaluate patient reported outcome measures using self-reported symptom severity and interference with daily activities using the MDASI-BT and/or MDASI-SP questionnaires.
- To measure symptom burden over the course of therapy to evaluate differences between patient's individual symptom severity, overall mean symptom severity, and difference in scores on the interference items between responders and non-responders.
- To describe the variability of symptom severity longitudinally over the treatment course and follow-up period.

1.1.3 Correlative Biology Objectives

1.1.3.1 Evaluate pretreatment tumor tissue for potential correlates of immune response including expression of immune suppressors, subtyping of Tumor Infiltrating

Lymphocytes (TILs) and molecular subtypes based on disease category (i.e., Wnt, SHH, Group 3, Group 4 for medulloblastoma).

1.1.3.2 Evaluate if there is a relationship between pretreatment immunocompetence defined by peripheral blood mononuclear cell assays and phenotyping correlates with response to nivolumab.

1.1.3.3 Determine if a change in immunocompetence over the course of the study treatment correlates with response and/or treatment-related toxicity.

1.2 BACKGROUND AND RATIONALE

1.2.1 Overview

The WHO Consensus Group publishes a monograph approximately every 7 years cataloging primary neoplasms of the central nervous system. The edition from 2016 recognizes over 130 central nervous system neoplasms, many with annual national (United States) incidences of less than 500 patients (1). To date, investigations for many of these cancers have been challenging as there has been limited funding and as a consequence, difficulties in generating a critical mass of investigators for these rare tumors. Fortunately, the National Institutes of Health is uniquely suited to pursue investigations for these rare cancers as evidenced by the rare diseases program at the National Cancer Institute (https://ccr.cancer.gov/research-rare-diseases). Recognizing that this is an unmet need, members of the NIH Brain Tumor Program have developed a Rare Central Nervous System Tumor Initiative to be able to study a variety of neoplasms. The resources of the NIH will be utilized to advance knowledge of these diseases. We have created a tissue repository with clinically annotated tumor tissues and genomic DNA. Tumor tissues from participating patients will also be collected and used for specific molecular testing for consideration of available clinical trials or as correlative evaluation as a component of assessment of an experimental regimen.

This study (17C0102) will include the following rare CNS tumors: Ependymoma, Medulloblastoma, Parenchymal Pineal Region Tumors (Pineoblastoma, Pineocytoma, Pineal Tumor of Intermediate Differentiation, Papillary Tumor of the Pineal Region), Choroid Plexus Tumors (Carcinoma, Papilloma, Atypical Papilloma), Histone Mutated Gliomas, Gliomatosis Cerebri, ATRT, Malignant/Atypical Meningioma, Gliosarcoma or Primary CNS Sarcoma, Pleomorphic Xanthoastrocytoma (PXA) and Anaplastic Pleomorphic Xanthoastrocytoma (APXA), and tumors formerly known as Primitive Neuro-Ectodermal Tumors (Embryonal Tumor with Multilayered Rosettes, Medulloepithelioma, CNS Neuroblastoma, CNS Ganglioneuroblastoma, CNS Embryonal Tumor NOS; and tumor entities emerging from methylation profiling of CNS-PNETs: CNS neuroblastoma with FOXR2 activation, CNS Ewing sarcoma family tumor with CIC alteration, CNS high-grade neuroepithelial tumor with MN1 alterations, and CNS high-grade neuroepithelial tumor with BCOR alteration). Given the rarity of each of the tumors, it is not surprising that there is a paucity of proven therapies. Most of these neoplasms are treated with maximum surgical resection followed by treatment with external beam radiotherapy. With few exceptions (medulloblastoma, ependymoma), there are no effective systemic regimens and even in chemotherapy sensitive disease, most patients with recurrence eventually have neither established salvage treatments available nor any ongoing clinical trials that allow their rare disease. In the setting of this unmet need, we propose to create a basket protocol that will evaluate the efficacy of the PD-1 inhibitor, nivolumab, in patients with

refractory rare central nervous system neoplasms. We plan to include adult patients (≥18 years of age).

1.2.2 Overview of the Tumor Types

1.2.2.1 Ependymoma

Ependymomas are a heterogenous group of primary central nervous system neoplasms with varied clinical characteristics, pathologic appearances, survival outcomes, cytogenetic, gene expression, and epigenetic profiles. According to the Central Brain Tumor Registry of the United States (CBTRUS), ependymomas make up 1.9% of all primary CNS tumors and 5.9% of all gliomas. To underscore how uncommon these tumors are, in the United States between 2004-2006, 2,147 ependymoma or anaplastic ependymomas and 798 ependymoma variants were reported (2). The World Health Organization (WHO) separates ependymal tumors into subependymomas (Grade 1), myxopapillary ependymomas (Grade 1), ependymoma (Grade 2) and anaplastic ependymoma (Grade 3)(1). As described below, the new WHO classification does recognize the molecular subcategorization of supratentorial ependymoma based on the presence of either the c11orf95-RELA fusion or the YAP1-MAMLD1 fusion.

Classically, the prognosis for patients with ependymomas was based on clinical factors including age of diagnosis, extent of tumor resection and although in pediatrics somewhat controversial, tumor grade by WHO criteria. More recent studies have clearly demonstrated that molecular subtypes exist that further refine the prognosis of ependymoma that provide additional prognostic information to the previously mentioned factors of age, tumor grade, anatomic location, and extent of surgical resection(3). Gene expression profiling has identified three distinct subtypes of posterior fossa ependymomas, with these subtypes being recapitulated in multiple studies(3-5). Ependymomas vary significantly by the CNS compartment of origin. Supratentorial tumors are distinct from both infratentorial and spinal cord tumors. The largest subtype of supratentorial ependymomas have a unique gene fusion, C11orf95-RELA, abbreviated as the RELA fusion(6). Loss of chromosome 9p, and CDK2NA/B deletion, previously associated with poor prognosis in supratentorial ependymomas, are found exclusively in tumors with the RELA fusion, as is chromothripsis (chromosome shattering)(3). The other main supratentorial ependymoma subtype has fusions in the YAP1 gene, mainly YAP1-MAMLD1, abbreviated as the YAP1 fusion(3, 6). Posterior fossa ependymomas have a subtype characterized by a hypermethylated genome (CIMP+; called Type A) that is found mainly in young children and portends a poor prognosis. Adolescents and adults typically have infratentorial tumors that do not have hypermethylation (CIMP-, called Type B)(5). Spinal cord tumors have not been as well characterized, although histologically they are separated into ependymoma (Grade II), anaplastic ependymoma (Grade III) and myxopapillary ependymoma, which while designated as WHO grade I, if not resected en-block will have a prognosis similar to Grade II spinal cord ependymoma(7). The myxopapillary ependymoma was recently found to have a unique metabolic signature by gene expression analysis(8). Finally, a new subtype of ependymoma originating in the spine, defined moleculary by the presence of MYCN amplification and with clinically aggressive behavior has been recently described (9-11).

The extent of surgical resection has been demonstrated to impact prognosis; therefore, maximal surgical resection, when feasible, is the standard of care for all ependymoma patients(7, 12, 13). For patients with anaplastic ependymoma, radiation is required. For lower grade ependymoma,

radiation is typically used if the resection is incomplete and for posterior fossa tumors in adults although prospective, randomized data do not exist(13), retrospective data suggest a marked advantage if radiation treatment is given even with complete resection. Platinum-containing chemotherapy regimens have the been traditionally used but have limited efficacy in adult patients for recurrent disease(14). A recently completed phase II clinical trial in adults with recurrent ependymomas suggested that a combination of temozolomide chemotherapy and targeting tyrosine kinases with lapatinib may be an effective treatment strategy(15). A significant proportion of ependymomas recur within 5 years of diagnosis and are recalcitrant to standard, cytotoxic chemotherapies that display modest activity at best in most ependymomas. Developing effective systemic treatments including targeted and immunologic therapeutic strategies is, perhaps, the most important step forward to improve clinical outcomes for ependymoma patients.

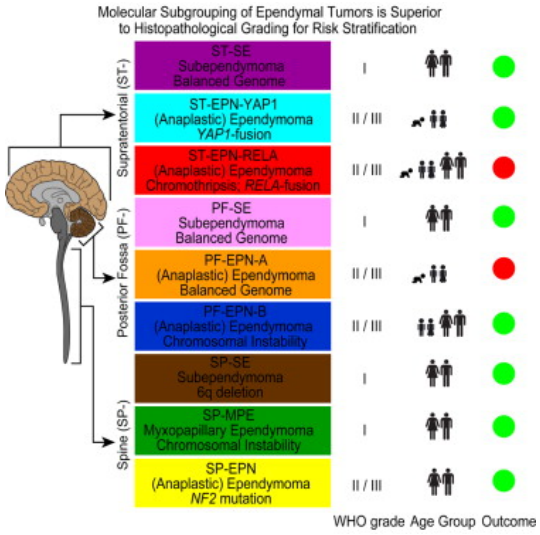


Figure 1: Molecular Subgrouping of Ependymal Tumors is Superior to Histopathological Grading for Risk Stratification

Although there have not been extensive studies of the immunophenotype of ependymoma, the well-defined molecular signatures of the subtypes with their unique fusion proteins (supratentorial disease) or hypermethylated phenotype (posterior fossa disease) provides an opportunity to test an immunotherapy regimen in these subgroups of a single disease entity.

1.2.2.2 Medulloblastoma

These infratentorial tumors are now classified by their molecular subtype. Five distinct subtypes have been defined since WHO 2016 (WNT; SHH p53 wild-type; SHH p53 mutated; and non-WNT/non-SHH, encompassing Group 3 and Group 4)(1). Adult medulloblastoma is most commonly characterized by sonic hedgehog (SHH) activation but with p53 wildtype. This is a medulloblastoma subgroup with variable prognosis. Long term survival is possible for patients with medulloblastoma, although once the disease recurs, it is incurable.

Other subtypes include SHH activated with p53 mutation, which is commonly associated with anaplastic features and portends a poor prognosis (typically seen in children and teenagers).

WNT activated is typically diagnosed in childhood with a good prognosis. Conversely, Group 3 and 4 do not have specific pathway activation but represent a pattern of gene expressions. Group 3 portends a poor prognosis and is thought to be exceptional in adults. Group 4 is second in frequency in adults, and prognosis seems worse than in children. A recent review found that in 201 adult patients with medulloblastoma, 62% were SHH, 28% were group 4 and 10% were WNT(16).

Conventionally, adult medulloblastoma was treated like the pediatric disease with the protocols being adapted for use in older patients. Unfortunately, these regimens are often poorly tolerated in adults and notably very few prospective trials have been completed. The recent development of smoothened (SMO) inhibitors such as vismodegib, may be a rational treatment for a subset of patients with SHH activated medulloblastoma. A recently published series demonstrated that there was a sustained objective response in approximately 10% of patients with SHH medulloblastoma treated with vismodegib (17). Overall, progression free survival was prolonged compared to historical controls, but ultimately there was relapse in nearly all cases, again underscoring the need for effective salvage regimens.

1.2.2.3 Parenchymal Pineal Region Tumors

Pineal parenchymal tumors (PPTs) include pineocytoma, pineal parenchymal tumor of intermediate differentiation, pineoblastoma and papillary tumor of the pineal region. These tumors are extraordinarily rare accounting for 0.1-0.3% of all primary CNS malignancies. Pineoblastoma, a WHO Grade IV tumor, makes up 45% of these tumors(1, 18). The majority of pineoblastomas occur in pediatric patients, and this tumor is extraordinarily rare in adults. Pineoblastomas appear similar histologically to other tumors formerly classified as primitive neuroectodermal tumors (PNETs) but differ with regards to response to treatment and genetically(19). Patients with no residual disease and without tumor dissemination in the CNS have superior outcomes. Most patients with residual and disseminated disease recur within five years, and have limited proven treatment options at tumor recurrence consistent with an overall 5-year progression-free survival rate of around 10%(20, 21).

PPT of intermediate differentiation are classified as WHO Grade II or III. In contrast to pineoblastomas, these tumors are more common in adults with the median age of onset in the early 40's. The tumor biology is quite variable, although these are more aggressive tumors with frequent recurrence and dissemination into the cerebrospinal fluid and drop metastases(22). Mitoses and proliferative indices appear to be major prognostic factors for these intermediate grade tumors(23). To date, surgical resection and radiation treatment remain the standard therapies with no proven effective chemotherapy salvage regimens. Finally, papillary tumor of the pineal region (PTPR) is a rare tumor first described in 2003 and added to the WHO classification in 2007. It is thought to originate from the ependymal cells of the subcommissural organ. Less than 200 cases have been published, and the median age at presentation is 32 years of age. Even though survival can be prolongued, local recurrences are common and CSF dissemination is possible. Management is heterogeneous and standard approach for newly diagnosed or recurrent tumors has not been clearly defined (24).

1.2.2.4 Choroid Plexus Tumors

Choroid plexus tumors (CPT) are rare neoplasms which account for less than 1% of all primary CNS neoplasms, but are most common in the first year of life (2, 25). Choroid plexus tumors are categorized as choroid plexus papillomas (CPPs, Grade I), atypical choroid plexus papillomas (Grade II), and choroid plexus carcinomas (CPC, WHO Grade III)(1). These tumors typically arise in the ventricles as they are thought to arise from the normal choroid plexus. CPPs occur more common in adults and most cases can be definitively treated with gross total resection; however, a subset designated as atypical are more likely to recur. Methylation profiling can separate CPCs from CPPs and atypical CPPs(25, 26). As with other CNS neoplasms, outcomes are better for patients able to undergo gross total resection and there is no dissemination of disease. There have been no established treatments developed for recurrent disease.

1.2.2.5 Chordoma

Effective with Amendment C (ver. 12/18/2019), this tumor type is not being included. This section is being retained for historical purposes.

Chordoma is a rare cancer which is classified as bone malignancy. It has an incidence rate of 0.1 per 100,000. Typical WHO grading has not been used and there is a discrepancy between the "low grade" and prognosis. Only 67% of patients survive 5 years and only 40% survive 10 years, pointing to a need to develop better therapies(27, 28). Chordomas are thought to have a notochord origin accounting for their midline location and distribution in the sacrum, clivus and spine(29). Chordomas are unique in the paucity of molecular finding, but the uniform duplication of the brachyury gene, a transcription factor important in normal notochord development. Chordomas are typically managed with maximal surgical resection and adjuvant radiotherapy, with proton radiotherapy used for skull base tumors, although fewer than 50% of these tumors can be completely resected. Given the high rate of recurrence after sub-total resection, there are several groups investing new systemic treatments including a vaccine strategy targeting brachyury at the NCI. Importantly, recent studies suggest that a subset of chordomas express PD-L1 and PD-L2(30, 31). These findings were confirmed by measurements performed by the NOB Immunology Program.

1.2.2.6 Gliomatosis cerebri

Gliomatosis cerebri (GC) is no longer classified as a separate entity since the WHO 2016 classification and has a behavior corresponding to a WHO grade III astrocytic neoplasm(1). GC is operationally defined as a diffuse glioma which displays extensive infiltration of the central nervous system, with involvement of at least three cerebral lobes, often with bilateral involvement of both hemispheres(32). Although GC can involve any portion of the CNS, it most commonly involves the cerebral hemispheres. GC has been reported in all age groups, but the peak incidence is around the 5th decade, similar to grade III gliomas(32).

The outcomes of patients with GC can be highly variable although overall the disease has a median survival of less than 2 years(32). Tumors with oligodendroglial components and with features of grade II tumors have a better prognosis. However, since the diagnosis is typically obtained via small biopsies, sampling may underestimate the tumor grade. Radiotherapy or chemotherapy may be used first-line for therapy in GC, however chemotherapy is often the frontline treatment to avoid administering whole brain radiotherapy. Given the rarity of the

disease, there have not been comparative trials evaluating the relative merits of radiation, chemotherapy or the combination. Treatment of recurrent or refractory disease follows the same paradigms for other gliomas with a series of cytotoxic chemotherapies, but these regimens often prove to be ineffective.

1.2.2.7 Brainstem glioma

Effective with Amendment C (ver. 12/18/2019), this tumor type is not being included. This section is being retained for historical purposes

Brainstem gliomas are rare, particularly in adults; accounting for less than 2% of gliomas. Although because of the location, these tumors are often not biopsied, they can range in grade from I to IV using WHO classification. Grade I tumors, typically pilocytic astrocytoma have a distinct appearance, being well circumscribed and often contrast enhancing. These tumors are distinct from the infiltrating gliomas. The histology of the infiltrating gliomas in the brainstem approximates that found in the supratentorial region in adults; astrocytomas, oligodendrogliomas, and glioblastomas. Interesting, the rate of IDH mutation seems to be lower than in comparable lower grade gliomas in the supratentorial region. Additionally, a relatively high percentage of brainstem glioblastoma have the EGFR viii mutation. Brainstem gliomas in adults have better outcomes compared to pediatric diffuse intrinsic pontine gliomas (DIPGs)(33-35). Whereas the average survival in pediatric DIPGs is uniform across studies with an average survival of 10-12 months with survival beyond 3 years being a rare event(36); in adults with infiltrating brainstem gliomas show an average survival of 30-40 months(34-37).

Management adults with brainstem gliomas is optimally determined by evaluation of tumor tissue. Glioblastoma may be best treated with concurrent temozolomide chemotherapy with radiation followed by maintenance temozolomide, emulating the treatment for supratentorial glioblastoma(37). However, this regimen may increase the risk of treatment-related brain injury so radiation therapy followed by maintenance chemotherapy may be preferable for lower grade brainstem gliomas. Brainstem-localized tumors are often excluded from clinical trials in adult high-grade glioma studies. Treatment for refractory or recurrent disease is limited once standard options, radiotherapy and cytotoxic chemotherapy have been exhausted. The rarity of these tumors, and especially the limitation of available tissue samples, makes immunophenotyping of any available archived samples of significant potential interest for therapeutic development.

1.2.2.8 Histone Mutated Gliomas (HMG)

This is a new entity and refers specifically in adults to tumors typically located in midline structures (pons, thalamus, corpus callosum, spinal cord) that have a specific molecular alteration, a K27M mutation in the H3F3A gene(1) and are classified as WHO grade IV. In adults with thalamic gliomas this is predominantly the H3.3 subtype and often associated with TP53 mutations, but mutually exclusive with IDH mutations(38). The K27M mutation has a variety of associated mutations that distinguish these tumors from “typical” infiltrating astrocytic neoplasms. Diagnosis is usually made by biopsying the lesion; given the midline location of the thalamus, resection is not feasible.

Overall, adults with thalamic gliomas including those with the K27M mutation have a better prognosis than patients with a similar K27M mutated brainstem glioma, for whom the median survival remains poor at 18-20 months(38, 39).

Although it is uncommon for HMGs to occur in non-midline locations, hemispheric HMGs have been identified associated with a different histone mutation - H3G34V; the prevalence of these tumors is still unknown and they have not been added yet to the WHO classification. Additionally, cases of non-midline H3K27M mutant gliomas have also been reported.

Standard adjuvant treatment after establishing a diagnosis of HMG typically involves local radiation. There is a lack of benefit of adjuvant temozolomide in the pediatric experience with DIPGs. Based on this observed lack of efficacy in pediatric patients and lack of any prospective or published experience in adult patients, concurrent and adjuvant temozolomide is considered of unclear benefit for HMG. Given the unique molecular features of HMGs and the limited treatment options, these genetically defined tumors will be included in this therapeutic protocol, regardless of their location (midline or hemispheric) and the specific histone mutation (H3K27M, H3G34V or any other histone mutations classified by genomic testing as variants of clinical significance).

1.2.2.9 Atypical Teratoid Rhabdoid Tumor (ATRT)

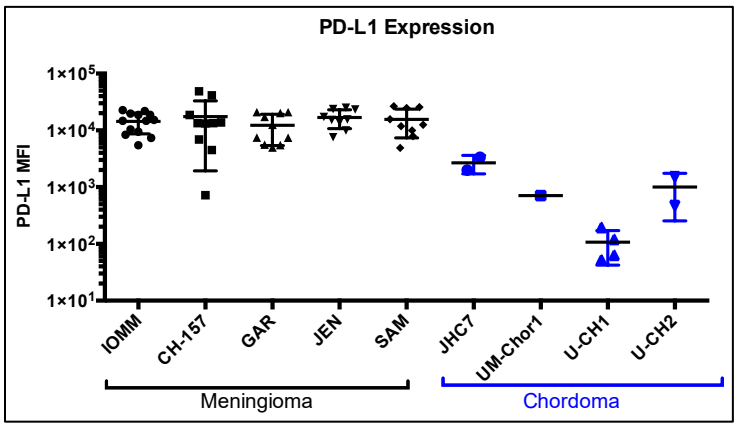
ATRT was first described in 1978 and thought to be a variant of Wilm's tumor of the central nervous system. This is a rare tumor in adults, but accounts for 10% of brain tumors in infants. In addition to the characteristic histologic appearance of rhabdoid cells, there is a characteristic mutation of INI1 which is diagnostic(40). The genetic form of the disease is characterized by the germline mutation of the INI1 (also known as SMARCB1). The genetic syndrome also includes soft tissue and kidney tumors. Treatment of ATRT comprises maximal surgical removal followed by radiation therapy(41). This can be regional unless there is evidence of CSF dissemination. Often radiation therapy is followed by intensive systemic chemotherapy, predominantly alkylating agents. Most patients have disease relapse within two years and salvage regimens rarely result in prolonged response. However, the prognosis is better for older children and adults where survival in years has been reported(41, 42). In a recent study including 89 pediatric patients with ATRT, ependymoma, high grade glioma and medulloblastoma, immunohistochemistry assays for PD-1 and PD-L1 were obtained, demonstrating PD-1 and PD-L1 expression in 35% and 40% of ATRT cases, respectively, therefore suggesting that this tumor might be a good candidate for PD-1 checkpoint blockade(43).

1.2.2.10 Malignant and atypical meningioma

Meningioma was first described by Harvey Cushing in 1922 and named for its apparent growth off of the meninges. WHO grading ranges from I to III, called low grade meningioma (Grade I), atypical meningioma (Grade II) and malignant meningioma (Grade III)(1). Histologic evaluation helps determine the tumor grade, however there are histologic subtypes that are designated Grade II and Grade III because of their well-established proclivity to recur and aggressive growth characteristics. For example, chordoid and clear cell meningioma are designated Grade II, whereas papillary and rhabdoid meningioma are classified as Grade III. Morphologic features that designate a Grade II meningioma include increased mitotic activity and brain invasion and at least 2 of the following features: increased cellularity, small cells with high nuclear to

cytoplasmic ratio and focal areas of necrosis. Grade III (malignant menigiomas) are characterized by overtly malignant histology, closely emulating sarcoma and markedly elevated mitotic activity.

The standard treatment for meningioma varies by WHO grade (reviewed in (44)). Surgical resection is the initial treatment of meningioma. Complete excision of Grade I meningiomas is often curative and even with partial resection, most patients are observed for progression before additional treatment is considered. Grade II meningiomas often warrant radiation treatment if there is visible residual disease; however, the use of radiation treatment in patients with extensive resection remains controversial. Grade III meningiomas always warrant post-operative radiation. Even with aggressive surgery and radiation, Grade III tumors often recur. There are no established treatments for patients with recurrent meningioma, although a variety of targeted agents, hormonal treatments and cytotoxic therapies have been attempted. Recently, extensive molecular analyses have uncovered some potential targets in selected tumors including AKT, SMO and NF2(45). An ongoing clinical trial is testing specific targeted agents based on these



mutations. Recent studies demonstrate that malignant meningiomas have elevated expression of PD-L1, making anti-PD-1 therapy a potentially effective treatment(46). These findings were also confirmed by studies in the NOB laboratory and shown in **Figure 2** below.

Figure 2: PD-L1 Expression in meningioma

1.2.2.11 Gliosarcoma and Primary CNS Sarcoma

The WHO classifies gliosarcoma (GS) as a variant of glioblastomas(1). GS often has a mixed histologic appearance with areas which have a typical glioblastoms appearance and a sarcomatous component in other areas (47). In some cases, the sarcomatous component can be the predominant morphology and the differentiation of a GS and primary central nervous system sarcoma can be difficult. GS is also often categorized as primary or secondary; tumors which at time of diagnosis have both features of glioblastoma and GS are considered primary GS whereas those tumors where the sarcomatous component was noted only at recurrence are considered secondary.(47, 48). Some GS have molecular finding frequently observed in glioblastoma including PTEN loss, p53 mutation, and p16 deletion, however EGFR amplification and IDH1 and 2 mutation are rare in GS(49). Unusual molecular findings for primary CNS cancers such as c-kit expression and genetic markers of epithelial to mesenchymal transition in other cancers have been described in GS(47).(49). Specific treatment strategies do not exist and current

standard treatment of GS is identical to glioblastoma with maximal surgical resection, radiotherapy with concurrent and adjuvant temozolomide for patients with a good clinical status, and regimens with a limited course of radiotherapy or temozolomide in poor prognosis patients (49).

Primary CNS sarcomas can include various sarcoma histologies including angiosarcoma, rhabdomyosarcoma, fibrosarcomas, and a significant proportion have histology that is sarcomatous but not otherwise classifiable(1). The actual prevalence of primary CNS sarcomas is unknown. Outside of surgical resection and radiotherapy, specific treatments for primary CNS or dural sarcomas do not exist. Chemotherapy regimens are typically based on the systemic sarcoma type and are likely to include chemotherapeutics with poor CNS penetration. Determining the immunophenotype may help generate hypotheses and guide future clinical trial design for primary CNS sarcomas.

1.2.2.12 Pleomorphic Xanthoastrocytoma (PXA) and Anaplastic Pleomorphic Xanthoastrocytoma (APXA)

PXA is a rare primary CNS tumor, representing less than 1% of all astrocytc neoplasms. PXAs most commonly affect children and young adults, with a median age at diagnosis of 22 years, although occurrence in older patients has also been reported. Despite its highly pleomorphic histological appearance, PXA has a relatively favorable prognosis compared with diffusely infiltrative astrocytoma. Recurrence-free survival at 5 years is 71% and overall survival 90%. PXA corresponds histologically to WHO grade II. BRAF V600E-mutant expression and loss of CDKN2A expression (secondary to homozygous deletion of *CDKN2A*) is frequently observed. *BRAF* point mutations (most V600E type) occur in approximately 50-78% of cases; however, they are not specific of PXA and are also observed in other primary CNS tumors (ganglioglioma, pilocytic astrocytoma). The presence of *BRAF V600E* mutation is not significantly different between pediatric and adult tumors, and it is unclear if it has prognostic implications. APXA corresponds histologically to WHO grade III and is defined by the presence of ≥ 5 mitoses per 10 high-power fields, and they have significantly worse survival (5 year overall survival 57%). The frequency of *BRAF V600E* mutations is lower than in PXAs (50).

Standard treatment for PXA is complete surgical resection. Additional nonsurgical therapies may be a consideration when treating patients with unresectable PXA or APXA. The role of adjuvant treatment for this tumor type, however, is not well established, and both chemotherapy and radiotherapy have been used mainly for APXA tumors with reports of good response. Recently, a small retrospective series reported adult patients with BRAF V600E mutant recurrent PXA that were radiation- and alkylator-refractory who were given salvage therapy using BRAF V600E inhibitor vemurafenib. Of the four patients evaluated, one had partial response, two had stable disease and one showed tumor progression (51). However, recurrence after treatment with radiation, standard chemotherapy or BRAF inhibitors is possible, and no previous studies have determined the immunophenotype and response to checkpoint inhibitors of recurrent PXAs and APXAs.

1.2.2.13 Tumors formerly known as Primitive Supratentorial Tumors (PNETs)

Primitive neuroectodermal tumors of the central nervous system (CNS-PNETs) are highly aggressive, poorly differentiated embryonal tumors occurring predominantly in young children

but also affecting adolescents and adults. Histologically, CNS-PNETs are characterized by small, poorly differentiated, or undifferentiated embryonal cells with a propensity for both glial and neuronal differentiation, but the neuropathological diagnosis is challenging due to a lack of defining molecular markers and histological overlap with other high-grade neuroepithelial tumors. As a result, the term PNET has been eliminated from the WHO classification of tumors of the CNS since 2016.

The original concept of PNET related medulloblastoma (i.e., PNET of the cerebellum) to embryonal tumors of the cerebrum (supratentorial PNET), but issues with the clinicopathological utility of classifying non-cerebellar CNS-PNETs have generated significant controversy over decades. This resulted in considerable uncertainty regarding accurate diagnosis and optimal treatment for affected patients. The 2007 WHO classification of CNS tumors listed CNS-PNET NOS (not otherwise specified) and four histological CNS-PNET variants distinguished by morphological features: CNS neuroblastoma, CNS ganglioneuroblastoma, medulloepithelioma (ME), and ependymoblastoma (EB). Embryonal tumors with abundant neuropil and true rosettes (ETANTR) have been recognized as a histological variant without a specific designation. The identification of focal amplification of a micro-RNA cluster on 19q13.42 (C19MC) as a unifying feature of ME, EB, and ETANTR (Eberhart et al., 2000; Korshunov et al., 2010; Korshunov et al., 2014; Li et al., 2009; Spence et al., 2014) led to the recognition of an overarching molecular and clinicopathological entity of embryonal tumors with multi-layered rosettes (ETMR, C19MC-altered) in the next revision of the WHO classification in 2016, adding to a growing list of defining molecular aberrations in high-grade pediatric CNS tumors. Thus, recent studies support the notion that CNS-PNETs represent a molecularly heterogeneous group of tumors, indicating an urgent need for better methods of classification.

As an example, a recent DNA methylation profiling study demonstrated that a significant proportion of institutionally diagnosed CNS-PNETs display molecular profiles indistinguishable from those of various other well-defined CNS tumor entities, and identified four new tumor entities from the remaining fraction of CNS-PNETS (CNS neuroblastoma with *FOXR2* activation, CNS Ewing sarcoma family tumor with *CIC* alteration, CNS high-grade neuroepithelial tumor with *MN1* alterations, and CNS high-grade neuroepithelial tumor with *BCOR* alteration) (52).

The understanding of embryonal tumors is undergoing changes, with an expectation that molecular markers could lead to more precise cataloging of these tumors and their subtypes. In the meanwhile, the 2016 CNS WHO has created a wastebasket category of CNS embryonal tumor, NOS that includes tumors previously designated as CNS PNET. Same as for other rare CNS tumors included in this protocol, no previous studies have determined the immunophenotype and response to checkpoint inhibitors in these tumors formerly known as PNETs.

1.2.3 PD-1 Blockade

Programmed death-1 (PD-1) has been recognized as a complementary checkpoint protein to CTLA-4 and is expressed on activated CD4+ and CD8+ T cells, B cells, monocytes, natural killer T (NKT) cells, and dentritic cells (DCs) (53). PD-1 signaling in T cells regulates immune responses by minimizing damage to bystander tissue and preventing the development of autoimmunity by promoting self-tolerance to self-antigens (54). Its major ligand PD-L1 (B7-H1)

is typically expressed on a subset of macrophages but can be induced by inflammatory cytokines in a variety of tissue types (55, 56). PD-L1 is also found on T cells, B cell, DCs, and mast cells (53). When activated T-cells expressing PD-1 encounter PD-L1, T-cell effector functions are subsequently diminished. The effector T cell functions including cytokine production, proliferation, and cell survival are inhibited as a result of the engagement of PD-1 to PD-L1. Tumor regression and prolonged host survival has been seen in preclinical studies of PD-1: PD-L1 blockade in murine tumor models. These promising results were noted in several tumor models including ovarian carcinoma, squamous cell carcinoma, melanoma, mastocytoma, hepatoma, and acute myeloid leukemia (57-59). Tumor regression was accompanied by increased effector T-cell function and cytokine production.

In patients with melanoma, high expression of PD-1 has been found on circulating T-cells and in tumor infiltrating T cells. Nivolumab is a fully humanized IgG4 monoclonal antibody that binds to the immune checkpoint molecule PD-1 with high affinity blocking its interaction with PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-1 blockade may lead to a reversal of tumor-induced T cell related immunosuppression (T-cell exhaustion). Nivolumab has been shown to block PD-1 and reverse the immunosuppression mediated by this molecule.

1.2.4 Nivolumab

The therapeutic benefit of nivolumab has also been established. In a phase I trial, single doses of nivolumab were found to induce rapid and durable responses in patients with solid tumors including melanoma, colorectal cancer, and renal cell carcinoma (60). Recent clinical trials have demonstrated activity of single-agent nivolumab in metastatic melanoma with a safety profile similar to that noted with ipiliumumab but with an overall lower incidence of adverse events and less frequent serious adverse events. A recent publication reported very few grade 3 or 4 adverse events even in patients with prior ipilimumab treatment (61). This study also demonstrated objective responses to single-agent nivolumab in patients who were either ipilimumab refractory (20%-30%) or ipilimumab naïve (~30%). They also found a correlation of response to tumor cell staining for the programmed death ligand 1 (PD-L1), but there were responses (20%-25%) even in patients without PD-L1 staining in tumor by immunohistochemistry. The promising clinical data for PD-1 blockade as an immunotherapy target extends its potential for use in other solid tumors beyond melanoma.

The use of an anti-PD-1 antibody has been tested in a preclinical model of glioblastoma (62). In this study, the glioma line GL261 was implanted intracranially and the animals were untreated, received radiation alone, received an anti-PD-1 antibody alone or received the combination. There was a statistically significant prolongation of survival in the combination arm and over the several experiments reported, long term survival (> 180 days) occurred in 15%-40% of the animals treated with the combination, but none of the other treatment arms.

Recent clinical data has validated the efficacy of nivolumab as monotherapy in the treatment of advanced melanoma. In a dose-escalation, cohort expansion study evaluating the antitumor activity and safety of nivolumab, 107 patients with advanced melanoma were enrolled between 2008-2012 (63). Nivolumab was administered intravenously once every 2 weeks in an outpatient setting in 8 week treatment cycles at 1, 3, or 10 mg/kg during dose escalation. After completion of dose escalation, each dose cohort was expanded to accrue approximately 16 patients with advanced melanoma. Patients with melanoma from any primary site including ocular with

measurable disease by RECIST (Response Evaluation Criteria in Solid Tumors) v1.0 (64) were permitted to enroll. Patients with brain metastases were eligible if lesions had been treated and were clinically stable for at least 8 weeks. Tumors were assessed radiographically following each treatment cycle with treatment continuing up to 96 weeks (12 cycles) until patients experienced a confirmed complete response, unacceptable toxicity, progressive disease, or until they withdrew consent. Progression Free Survival was found to compare favorably with that in the literature of similar patient populations. Median progression free survival was 3.7 months (95% CI, 1.9 to 9.1 months) with 1 and 2 year progression free survival rates of 36% (95% CI, 27% to 46%) and 27% (95% CI, 17% to 36%) respectively (63). Objective responses were seen in 31% of patients (33 of 107) with melanoma with an additional 7% (7 of 107) experiencing stable disease for 24 weeks or more. Durable responses were observed across all nivolumab doses tested within a 2-log range (0.1 to 10 mg/kg). Responses were durable and lasted beyond drug discontinuation.

The maximum-tolerated dose of nivolumab was not reached within the tested dose range. The most common adverse events of any grade that occurred in patients were fatigue (32%), rash (23%) and diarrhea (18%). Twenty-four (22%) of the 107 patients experienced a grade 3 to 4 treatment-related adverse event. Treatment-related select adverse events of any grade were observed in 58 (54%) of 107 patients the most common of which included skin disorders (36%), GI events (18%), and endocrinopathies (13%). There were no drug-related deaths in the population of patients with melanoma (63).

Nivolumab has demonstrated durable responses as monotherapy in several tumor types as described in the Investigator's Brochure. In confirmatory trials, nivolumab demonstrated significant clinical benefit as monotherapy, and in combination with other therapeutics.

The safety experience from clinical trials with nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in more than 32,000 subjects. For monotherapy, the safety profile is similar across tumor types. In Phase 3 controlled studies, the safety profile of nivolumab monotherapy is acceptable in the context of the observed clinical efficacy, and manageable using established safety guidelines. Clinically relevant AEs typical of stimulation of the immune system were infrequent and manageable by delaying or stopping nivolumab treatment and timely immunosuppressive therapy or other supportive care. Based on data from a completed Phase 1b study and a Phase 1/2 study, single doses of nivolumab did not result in unexpected safety findings for participants with sepsis or septic shock.

Moreover, a safety study was performed evaluating nivolumab as a single agent in patients with recurrent high-grade glioma. These results were reported at ASCO in 2016. (65) A total of 10 patients received nivolumab monotherapy at a dose of 3 mg/kg every 2 weeks. The median age was 59 and most had a good performance status (7 with KPS = 90, 1 with KPS = 80, 2 with KPS = 70). Treatment was well tolerated. As outlined in the Table **1** below, there were no grade 3 or 4 toxicities associated with the nivolumab treatment. Although there were a variety of adverse events reported, none altered treatment delivery.

Table 1 : Toxicities associated with the nivolumab treatment

	Nivolumab 3 mg/kg (n = 10)	
	Any grade	Grade 3–4
Patients with any TRAE, n (%)	9 (90)	0
ALT increased	0	0
Amylase increased	1 (10)	0
AST increased	0	0
Asthenia	0	0
Colitis	0	0
Confusional state	1 (10)	0
Cough	0	0
Decreased appetite	0	0
Diarrhea	1 (10)	0
Dizziness	1 (10)	0
Fatigue	3 (30)	0
Gait disturbance	2 (20)	0
Headache	2 (20)	0
Hyperthyroidism	1 (10)	0
Hypothyroidism	2 (20)	0
Lipase increased	2 (20)	0
Maculopapular rash	0	0
Nausea	3 (30)	0
Optic nerve disorder	2 (20)	0
Platelet count decreased	0	0
Pruritus	2 (20)	0
Pyrexia	0	0
Rash	2 (20)	0
Vomiting	1 (10)	0

1.2.5 Rationale for study cohorts (heavily pretreated and not heavily pretreated)

This study was originally designed to enroll patients into 4 different cohorts based on histological diagnosis (Ependymoma, High Grade Meningioma, Chordoma, Others). Current evidence in other cancers (melanoma) suggests that extensive prior chemotherapy alters the microenvironment and makes tumors less sensitive to immunotherapy (Hugo, W et al 2016, Cell 165:35-44; Hugo, W et al 2015 Cell 162: 1271-1285). Additionally, smaller tumor burden (more commonly found in non-heavily pretreated tumors) has been shown to be associated with better progression free survival and overall survival in other cancers (melanoma, NSCLC; Joseph et al 2018 Clin Cancer Res, Katsurada et al 2019 Anticancer Research). Together, these data suggest the potential for a different pattern of response in heavily pretreated versus non heavily pretreated patients, and we will aim to determine efficacy of this regimen separately in these two different disease settings across different tumor types.

1.2.6 Rationale for the patient-reported outcomes

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 and M.D. Anderson Symptom Inventory-Spine Tumor Module (MDASI-BT and MDASI-SP, respectively) allow the self-

reporting of symptom severity and interference with daily activities. The MDASI-BT and MDASI-SP have demonstrated reliability and validity in the adult primary brain and spine tumor patient population (66, 67). 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 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.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Histopathologically proven diagnosis of Ependymoma, Medulloblastoma, Parenchymal Pineal Region Tumors (Pineoblastoma, Pineocytoma, Pineal Tumor of Intermediate Differentiation, Papillary Tumor of the Pineal Region), Choroid Plexus Tumors (Carcinoma, Papilloma, Atypical Papilloma), Histone Mutated Gliomas, Gliomatosis Cerebri, ATRT, Malignant/Atypical Meningioma*, Gliosarcoma or Primary CNS Sarcoma, Pleomorphic Xanthoastrocytoma (PXA) and Anaplastic Pleomorphic Xanthoastrocytoma (APXA), and tumors formerly known as Primitive Neuro-Ectodermal Tumors (Embryonal Tumor with Multilayered Rosettes, Medulloepithelioma, CNS Neuroblastoma, CNS Ganglioneuroblastoma, CNS Embryonal Tumor NOS; and tumor entities emerging from methylation profiling of CNS-PNETs: CNS neuroblastoma with FOXR2 activation, CNS Ewing sarcoma family tumor with CIC alteration, CNS high-grade neuroepithelial tumor with MN1 alterations, and CNS high-grade neuroepithelial tumor with BCOR alteration) prior to registration.

*Individuals with extra CNS metastases from meningioma will be eligible even if pathology review fails to demonstrate high grade features on available tumor samples.

2.1.1.2 The tumor tissue (e.g., block or 20 unstained slides) must be available to be sent for immunophenotyping by NCI Laboratory of Pathology (See Section **16.6**).

2.1.1.3 Individuals must have progressive tumor growth after having received established standard of care and/or other experimental treatments for their newly diagnosed or recurrent disease. Individuals will be enrolled into 2 different cohorts (cohort 1 or heavily pretreated; cohort 2 or not heavily pretreated; see 2.4).

2.1.1.4 Age ≥ 18

2.1.1.5 Karnofsky performance status ≥ 70 (See Section **16.1**) within 14 days prior to Step 2 registration; Individuals with severe paraparesis/paraplegia who need minimal assistance for selfcare due to their motor deficit but are otherwise functionally independent will be considered eligible.

2.1.1.6 Adequate hematologic function based on CBC/differential within 14 days prior to Step 2 registration defined as follows:

- Absolute neutrophil count ≥ 1,500 cells/mm3;
- Platelet count ≥ 100,000 cells/mm3
- Hemoglobin > 9.0 g/dl (may be transfused to achieve this level)

2.1.1.7 Adequate renal function within 14 days prior to Step 2 registration defined as follows:

- BUN ≤ 30 mg/dl and
- Serum creatinine ≤ 1.7 mg/dl

Note: If the serum creatinine is greater than 1.7 mg/dl, a 24-hour urine creatinine clearance will be obtained and if the result of this study is within normal limits*, the patient would be eligible to enroll onto study. (*Normal Creatinine Clearance Range: Male: 90 - 130 ml/min; Female: 80 - 125 ml/min)

2.1.1.8 Adequate hepatic function within 14 days prior to Step 2 registration defined as follows:

- 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 and
- ALT **and** AST ≤ 2.5x ULN

2.1.1.9 No active or chronic hepatitis infection. HCV antibody (for Hepatitis C) and Hepatitis B Surface antigen and Hepatitis B core antibody must be negative. This has been routinely incorporated into immunotherapy trials with checkpoint inhibitors because of concerns that the risk of treatment-induced hepatic injury is increased in the setting of active viral hepatitis.

2.1.1.9.1 The individual must not be on a corticosteroid dose greater than physiologic replacement dosing defined as 30 mg of cortisone per day or its equivalent.

2.1.1.9.2 The individual must provide study-specific informed consent prior to study entry. No Durable Power of Attorney or Next of Kin can provide initial consent.

2.1.1.10 The effects of *nivolumab* on the developing human fetus are unknown. For this reason, women of childbearing potential (WOCBP) must use appropriate method(s) of contraception. *WOCBP should use an adequate method to avoid pregnancy for 5 months (30 days plus the time required for nivolumab to undergo five half-lives) after the last dose of investigational drug.*

NOTE: Based on the evidence cited in Nivolumab IB ver. 20, given that nivolumab is not a genotoxic agent, and that relevant systemic concentrations sufficient to produce a risk of fetal toxicity are not expected in IOCBP partners from exposure to an individual's seminal fluid, men that can father children will not be required to use contraceptive measures and/or a latex or other synthetic condom during sexual activity with an WOCBP partner.

2.1.2 Exclusion Criteria

2.1.2.1 Individuals who are receiving any other investigational agents.

2.1.2.2 Prior use of an immunotherapy such as (but not limited to) a vaccine therapy, dendritic cell vaccine, other checkpoint inhibitors, or intracavitary or convectional enhanced delivery of chemotherapy.

2.1.2.3 Prior or concurrent malignancy unless its natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen.

2.1.2.4 Severe, active co-morbidity defined as follows:

- Unstable angina within the last 6 months prior to Step 2 registration.
- Transmural myocardial infarction within the last 6 months prior to Step 2 registration.
- Evidence of recent myocardial infarction or ischemia by the findings of S-T elevations of ≥ 2 mm using the analysis of an EKG performed within 14 days prior to Step 2 registration.
- New York Heart Association grade II or greater congestive heart failure requiring hospitalization within 12 months prior to Step 2 registration.
- History of stroke, cerebral vascular accident (CVA) or transient ischemic attack within 6 months prior to Step 2 registration, with the exception of pericavitary ischemia due to tumor resection.
- Serious and inadequately controlled cardiac arrhythmia.
- Significant vascular disease (e.g., aortic aneurysm, history of aortic dissection) or clinically significant peripheral vascular disease.
- Evidence of bleeding diathesis or coagulopathy.
- Serious or non-healing wound, ulcer, or bone fracture or history of abdominal fistula, gastrointestinal perforation, intra-abdominal abscess, major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to Step 2 registration, with the exception of the craniotomy for tumor resection.
- Acute bacterial or fungal infection requiring intravenous antibiotics at the time of registration.
- Chronic obstructive pulmonary disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at the time of registration.
- Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects.
- Known 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 participants with AIDS is based on the lack of information regarding the safety of nivolumab in patients with active HIV infection.

- Active connective tissue disorders, such as lupus or scleroderma, which in the opinion of the treating physician may put the patient at high risk for immunologic toxicity.

2.1.2.5 Individuals 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, should be excluded. These include but are not limited to individuals 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 individuals with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome should be excluded because of the risk of recurrence or exacerbation of disease.

Of note, individuals with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Participants 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. However, individuals with vitiligo, diabetes mellitus, and Hashimoto thyroiditis on appropriate replacement therapy may be enrolled.

2.1.2.6 Any other major medical illnesses or psychiatric impairments that in the investigator's opinion will prevent administration or completion of protocol therapy.

2.1.2.7 Allergies and Adverse Drug Reaction: History of allergy to study drug components.

2.1.2.8 Pregnancy or lactating women due to possible adverse effects on the developing fetus or infant due to study drug. Women of childbearing potential must have a negative serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to Step 2 registration.

2.1.2.9 History of severe hypersensitivity reaction to any monoclonal antibody.

2.1.2.10 Individuals unable to have MRIs.

2.2 RECRUITMENT STRATEGIES

This protocol may be abstracted into a plain language announcement posted on NIH websites, including www.clinicaltrials.gov and the CCR website, and on NIH social media platforms. Outside providers and colleagues may directly refer patients for screening into this study. Participating Site recruitment efforts must be consistent with the multicenter protocol and approved by the local IRB.

2.3 SCREENING EVALUATION

2.3.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the participant 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 **12.7.2**.

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

The following activities will be performed only after the subject has signed the consent for this study. Assessments performed at other clinical facilities or on another protocol at enrolling site within the timeframes below may also be used to determine eligibility once an individual has signed the consent. Individuals will be required to have the following studies performed for eligibility. The maximum time before registration for each study is provided.

Study	Maximum allowable time before Step 2 registration
MRI of involved area (Brain and/or Spine)	28 days
Body CT scans (if systemic disease present) ***Note**: CT scans will not be acceptable for evaluation of CNS disease	28 days (if applicable)
Complete Blood Count	14 days
Hepatic and renal function (ALT, AST, bilirubin, serum creatine, BUN; 24-hour urine for creatine may be collected if required per section **2.1.1.7**)	14 days
Serum pregnancy test	24 Hours
Assessment of Karnofsky Performance Status	14 days
Confirmation of corticosteroid dose being zero or physiologic replacement	7 days
Confirmation of histopathology (**non-NIH participating sites**) *+ • *Outside Pathology report* • *Tumor tissue available for review to confirm morphologic diagnosis* • *Tumor tissue (e.g., block or 20 unstained slides) available for molecular and immune profiling*	Outside pathology report must be available and tumor tissue must be submitted anytime, before Step 2 registration. Tumor tissue review to confirm diagnosis by NCI Laboratory of Pathology will NOT need to be completed before Step 2 registration. If the outside pathology report does not provide an unequivocal diagnosis, central NIH review is mandatory before Step 2 registration.
Confirmation of histopathology (**NIH site**) * • *Tumor tissue available for review to confirm morphologic diagnosis*	Confirmation of diagnosis by NCI Laboratory of Pathology will be required for registration (to be completed any time before Step 2).

Study	Maximum allowable time before Step 2 registration
• *Tumor tissue (e.g., block or 20 unstained slides) available for molecular and immune profiling*	
EKG	14 days
HCV antibody, HBV surface antigen (or HBV DNA Quantitative), and HBV core antibody	14 days

[+]Individuals with extra CNS metastases from meningioma will be eligible even if pathology review fails to demonstrate high grade features on available tumor samples.

2.4 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

2.4.1 NCI/NOB Registration

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://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit.

2.4.2 For NCI-CONNECT Participating Site Registration

Registration will be a two-part process as participants are screened on this protocol. A protocol registration form will be supplied by the NCI-CONNECT Research Nurse Coordinator and updates will be provided as needed. To initially register a participant, after the participant has signed consent, complete, the top portion of the form and send it to the NCI-CONNECT Research Nurse Coordinator. Once eligibility is confirmed, after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the participant is being registered for treatment and send to the NCI-CONNECT Research Nurse Coordinator.

In addition, source documents supporting the eligibility criteria must be sent to the NCI-CONNECT Research Nurse Coordinator. The NCI-CONNECT Research Nurse Coordinator will notify you either by e-mail, or phone, that the protocol registration form has been received which will include the unique patient/subject ID number. Questions about eligibility should be directed to the NCI-CONNECT Research Nurse Coordinator or PI. Questions related to registration should be directed to the NCI-CONNECT Research Nurse Coordinator.

When a participant has a status change (e.g., subject screened on the study, does not meet eligibility criteria, and is removed from the study, participant is taken off protocol therapy or off study, etc.), the Participant Status Update Form will be supplied by the NCI-CONNECT Research Nurse Coordinator. Send the completed form to the NCI-CONNECT Research Nurse Coordinator via Secure Email and File Transfer (SEFT) .

2.4.3 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 and eligibility criteria.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a reversible medical or surgical complication or a decision to proceed with another therapy may be rescreened once the underlying condition has resolved or they experience further tumor progression.

2.4.4 Treatment Assignment Procedure:

Cohorts

Number	Name	Description
1	Heavily pretreated	Participants with 3 or more prior therapies* (radiotherapy and/or standard or investigational antineoplastic drugs#)
2	Non-heavily pretreated	Participants with up to 2 prior therapies* (radiotherapy and/or standard or investigational antineoplastic drugs#)

*resection alone will not count as prior therapy for the purpose of cohort assignment in this trial
#standard and/or investigational drugs given as part of multiagent therapy will be counted individually

Arm

Number	Name	Description
1	Experimental Therapy	Participants will be treated with the immune checkpoint inhibitor, nivolumab, at a standard dose of 240 mg intravenously every 2 weeks (+/- 3 days) for cycles 1 through 2, then doses of 480 mg every 4 weeks (+/- 3 days) for a total of 14 additional doses (cycles).

Arm Assignment

There will be no randomization. Participants will be assigned into 2 cohorts (cohort 1, heavily pretreated; cohort 2, non-heavily pretreated). All patients in both cohorts will be assigned to the same treatment arm (arm 1).

2.5 BASELINE EVALUATIONS

The following table outlines the Baseline Assessments:

Assessment	Timeframe (calendar days)
	Within 14 days prior to treatment (Unless otherwise noted)
Karnofsky performance status	X
Contrast-enhanced MRI of involved area (Brain and/or Spine) and/or Body CT scans (if systemic disease present) [1]	X
CBC/diff, ANC, platelets	X
BUN, serum creatinine; creatinine clearance if needed (see 2.1.1.7)	X
Total bilirubin, ALT, AST	X
Serum pregnancy test (if the patient is of child-bearing potential)	within 24 hours prior to treatment
EKG	X
History and physical	X
CD4 Count	X
Blood for Immunologic Monitoring	X
Endocrine Function: Fasting morning cortisol and TSH	X
MDASI-BT and/or MDASI-SP[2]	X

[1] The baseline imaging does not have to be repeated if done within 28 days of C1D1.

[2]The MDASI-BT and/or MDASI-SP (Appendices **16.1**) will be administered at time of baseline imaging or up to 14 days prior to treatment initiation. The choice of instrument will be based on the location of the index lesion for study enrollment. This may be in the brain (MDASI-BT), spine (MDASI-SP) or both (completing both instruments if lesions in the brain and spine are being followed for response). Even if only extra CNS disease is present at the time of trial enrollment, the appropriate MDASI questionnaires will be used based on the location of the tumor at the time of initial diagnosis.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is an open label phase II clinical trial. Participants will be treated with the immune checkpoint inhibitor, nivolumab, at a standard dose of 240 mg intravenously every 2 weeks (+/- 3 days) for cycles 1 through 2, then doses of 480 mg every 4 weeks (+/- 3 days) for a total of 14 additional doses (cycles). A cycle will be defined as 4 weeks and patients will undergo efficacy assessments using imaging every 2 cycles. Toxicity assessments will occur before the initiation of each cycle. Patient outcomes measures (PROs) (MDASI-BT and/or MDASI-SP) will be completed within 14 days prior to initiating treatment (baseline) and at the time of each imaging study (every 2 cycles) but prior to the participant being informed of the imaging results.

After completion of the planned treatment course or if treatment was stopped because of toxicity, participants will undergo imaging evaluations and PRO measurements every 8 weeks (or 2

months) for one year, then every 3 months for the next year, then every 4 months for the next year and then every 6 months while the participant remains on the protocol.

3.2 DRUG ADMINISTRATION

Participants will be treated with the immune checkpoint inhibitor, nivolumab, at a standard dose of 240 mg intravenously every 2 weeks (+/- 3 days) for cycles 1 through 2, then doses of 480 mg every 4 weeks (+/- 3 days) for a total of 14 additional doses (cycles). Treatment will be routinely administered on an outpatient basis. Nivolumab will be administered through IV infusion over 30 minutes at a flat dose of 240 mg or 480 mg. Based on patient tolerability, a slightly longer administration time of between 30 and 60 min is allowed. The Investigator's Brochure shows no significant differences in safety/efficacy between 30-and 60-minute infusion administration times. See Section **14.1.12** for more details about study agent administration. Vital signs will be measured within 1 hour before and after drug infusion.

This treatment is intended to be given on an outpatient basis but can be given on an inpatient basis. Nivolumab is administered through a 0.2 micron to 1.2 micron pore size, low-protein binding in-line filter.

A cycle will be defined as 4 weeks and patients will undergo efficacy assessments using imaging every 2 cycles.

3.3 DOSE MODIFICATIONS

Participants in this study will be considered moderately/severely immunocompromised given their diagnosis of a tumor and the experimental nature of this therapy and potential effects on immune function. Participants will be strongly recommended to follow current CDC guidelines for vaccination against COVID and other viruses and following other infection control measures (i.e., mask use, social distancing, etc.) for moderately/severely immunocompromised individuals:

https://www.cdc.gov/covid/vaccines/immunocompromised-people.html

https://www.cdc.gov/cancer/initiatives/covid-19.html?CDC_AAref_Val=https://www.cdc.gov/cancer/dcpc/about/covid-19.htm

There are no planned dose reductions for nivolumab. The tables below outline the criteria for delaying (then restarting) or stopping treatment by toxicity type.

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab**
Grade 1	Continue treatment. No change in dose *. (See **16.4**)
Grade 2	Hold* until ≤ Grade 1 (toxicity resolved). Consider steroid treatment if persists > 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
*Participants with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphagoid. Pruritus may occur with or without skin rash and should be treated	

Skin Rash and Oral Lesions	**Management/Next Dose for Nivolumab**
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 **Skin Adverse Event Management Algorithm** (See **16.4**)	

Liver Function AST/ALT/T. Bili *	**Management/Next Dose for Nivolumab**
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. (See **16.4**)
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 Hepatitic Adverse Event management algorithm (See **16.4**)	
* Total bilirubin not used for determining AEs in patients with Gilbert syndrome.	

Renal Function Serum Creatinine	**Management/Next Dose for Nivolumab**
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 Adverse Event management algorithm (See **16.4**)	

Diarrhea/ Colitis	**Management/Next Dose for Nivolumab**
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. (See **16.4)**
Grade 3	Off protocol therapy.
Grade 4	Off protocol therapy.
Participants with grade 2 symptoms but normal colonoscopy and biopsies may be retreated after resolution. Participants 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.	

Diarrhea/ Colitis	**Management/Next Dose for Nivolumab**
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 Adverse Event management Algorithm (See **16.4**).	

Pancreatitis Amylase/Lipase	**Management/Next Dose for Nivolumab**
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.
Grade 3	Discontinue treatment.
Grade 4	Off protocol therapy.
Participants may develop symptomatic and radiologic evidence of pancreatitis as well as DM and diabetic ketoacidosis. 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 participants 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 **16.4**).	

Pneumonitis	**Management/Next Dose for Nivolumab**
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 participants 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. Participants 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 (See **16.4**).	

Other GI nausea-vomiting	**Management/Next Dose for Nivolumab**
Grade 1	No change in dose.

Other GI nausea-vomiting	**Management/Next Dose for Nivolumab**
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 participants should go off protocol therapy
Grade 4	Off protocol therapy
Participants with grade 2 or 3 N-V should be evaluated for upper GI inflammation and other immune related events.	

Fatigue	**Management/Next Dose for Nivolumab**
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/Next Dose for Nivolumab**
Grade 1	Continue treatment; continue close observation.
Grade 2	Hold dose pending evaluation and observation. Hold until baseline. *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
*Participants 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 study.	
Recommended management: See Neurologic Adverse Event Management Algorithm (See **16.4**)	

*These guidelines DO NOT include neurological worsening due to suspected pseudoprogression secondary to checkpoint inhibitor therapy (i.e., treatment effect such as increased edema and/or enhancement); which will be managed as per treating physician and PI discretion (see also 3.8.2)

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab**
Grade 1	Asymptomatic TSH elevation *Treatment can continue. endocrine consult recommended
Grade 2	Hold until patients are on a stable replacement hormone regimen. If treated with steroids participants must be stable off steroids for two weeks. Resume at same dose level.

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab**
Grade 3	Off study treatment.
Grade 4	Off protocol therapy
Note all participants 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. Please evaluate pituitary function before beginning steroid therapy or replacement therapy of any kind. *Note participants with thyroiditis may be retreated on replacement therapy. Participants must be evaluated to rule out pituitary disease prior to initiating thyroid replacement.	
Recommended management: See Endocrine Management Algorithm (See **16.4**)	

Fever	**Management/Next Dose for Nivolumab**
Grade 1	Symptomatic treatment with analgesics
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 treatment
Participants 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**	**Management/Next Dose for Nivolumab**
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, respectively, may not require discontinuation after discussion with and approval from the overall PI.
** **Not agent related**, or agent related non-immunologically mediated	
Recommended management: As clinically indicated	

ALL OTHER EVENTS: IMMUNE RELATED	**Management/Next Dose for Nivolumab**
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	Hold** until ≤ Grade 1 continue at investigator discretion, with the following exceptions: Grade 3 lymphopenia or asymptomatic amylase or lipase does not require dose delay Grade 3 uveitis, pneumonitis, bronchospasm, thrombocytopenia lasting >7 days or associated with bleeding, hypersensitivity reaction, or infusion reaction of any duration requires 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

ALL OTHER EVENTS: IMMUNE RELATED	**Management/Next Dose for Nivolumab**
	– 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, respectively, may not require discontinuation after discussion with and approval from the overall PI.
Recommended management: As clinically indicated.	

3.4 QUESTIONNAIRES (APPENDIX 16.1)

The MDASI-BT or MDASI-SP will be administered at baseline and at the time of imaging analysis as outlined in the protocol. Of note, every attempt should be made to administer the MDASI-BT and/or MDASI-SP prior to informing the participants about the results of the imaging study. The MDASI-BT or MDASI-SP will be completed only by the participant, unless changes in vision or weakness make this difficult. If this occurs, then the caregiver or research assistant may read the questions to the participant or assist with marking the severity number or score as described by the participant. A caregiver may complete the questionnaires as a participant proxy if the participant's deficits preclude self-report; however, this must be done at every assessment from baseline to end of treatment. Full instruments are provided in the Appendix **16.1**. In addition, information regarding demographics and treatment history will be collected as part of the larger study and used in this analysis.

The MDASI-BT and MDASI-SP consist 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, and those associated with the underlying disease. The questionnaire also includes ratings of how much symptoms interfered with different aspects of a participant's life in the last 24 hours. These interference items include general activity, mood, work (includes both work 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 and MDASI-SP have been translated into multiple languages, but the English language version will be used for this study (67).

3.5 TELEHEALTH VISITS

In the extenuating event that a subject cannot travel to be seen in person at the study site due to a crisis causing travel and staffing restriction, the Provider Assessment may occur via telehealth visits. Telehealth visits (synonyms: telemedicine, videoconferencing, virtual visits, video phone call or remote visits) will be allowed under these extenuating circumstances for patients on-study unless these visits are essential to maintaining patient safety while on-study. Decisions regarding the use of telehealth will be based on the patient's physician's decision regarding the risk of an in-person clinic visit compared with the potential benefit of a comprehensive in-person

evaluation. The medical record or progress note for the telehealth visit should state that the risks were greater than the benefit.

Telehealth visits for protocol assessments will be conducted in compliance per NIH **HRPP Policy 303**. For example, to ensure privacy, the patients will confirm their identities before the interaction or exchange of private information begins. In addition, documentation of telehealth visits must comply with local requirements (e.g., date and time of the real-time video interaction, location of the subject, individuals present, assessments performed, etc.).

General interviews may be conducted adequately via telehealth visits. For example, to obtain a patient's medical history, check-in with a participant regarding their current health status, or to administer a questionnaire to a participant related to their level of functioning or activities of daily living.

Clinical assessments via telehealth visits for patients on study will not compromise either the primary or secondary objectives and will likely have minimal impact on the ability to complete this study as long as administration of treatment (e.g., nivolumab) is not compromised.

Furthermore, a patient may be referred to another local provider or asked to come to the NIH Clinical Center for an in-person assessment, if clinically indicated, and at the discretion of the investigator. In the case of any visits with participants' local providers, records including outside labs will be obtained for the research records.

Given that the study requires only standard, clinical laboratory assessments and imaging per routine radiologic parameters/procedures, any possible interlaboratory or facility variability is not expected to be a concern. If there is any question about possible progression, a patient may be asked to have repeat imaging and/or additional assessments to confirm response.

The following clinical assessments generally obtained during a physical examination (i.e., H&P) can be conducted via telehealth visits through discussion and remote/video observation:

- Karnofsky Performance Status.
- Neurological exam to assess cognition remotely will use the American Academy of Neurology Telemedicine guidance document for evaluation of memory, attention, concentration, language, eye movements, facial weakness, strength, walking and coordination.
- Physical exam to observe rashes, thrust, surgical scar, and swelling will use the American Academy of Dermatology Teledermatology guidance document for physical evaluation during telehealth visits.

The following clinical assessments are NOT feasible via telehealth visits and will not be required:

- Hearing and vision
- Listening to heart and lungs
- Assessing weakness
- Checking reflexes
- Checking for abnormalities in sensation

- Feeling lymph nodes, areas of swelling
- Blood correlatives tests

3.6 STUDY CALENDAR

	Screening[13]/ Baseline[1]	**During Active treatment*** **		**Post Therapy Follow-up**	
		Day 1 (- 3 d) of each cycle (1 cycle is 4 weeks)	Within 7 days of the initiation of odd numbered cycles (1, 3, 5, etc.)[12]	From the last dose of nivolumab (+/- 1 week), q 2 months x 6, q 3 months x 4, then q 4 months x 3 then q 6 months[#]	1 month (+/- 1 week) after the last dose of nivolumab, then at the discretion of the treating physician for 5 years[#]
History and physical	X		X[11]		
Submission of tumor tissue (e.g., block or 20 unstained slides)[4]	X				
EKG	X				
Karnofsky performance status	X			X	
CBC/diff, ANC, platelets	X	X[10]			X
BUN, serum creatinine; and if required at screening only creatinine clearance, See section (2.1.1.7)	X	X[10]			X
Total bilirubin, AST, ALT	X	X[10]			X
HCV antibody, HBc antibody and HBV surface antigen*	X				
CD4 count[5]	X[2]	X[5, 10]			X[5]

	Screening[13]/ Baseline[1]	**During Active treatment*** **		**Post Therapy Follow-up**	
		Day 1 (- 3 d) of each cycle (1 cycle is 4 weeks)	Within 7 days of the initiation of odd numbered cycles (1, 3, 5, etc.)[12]	From the last dose of nivolumab (+/- 1 week), q 2 months x 6, q 3 months x 4, then q 4 months x 3 then q 6 months[#]	1 month (+/- 1 week) after the last dose of nivolumab, then at the discretion of the treating physician for 5 years[#]
Blood for immunologic monitoring[6]	X[2]		X[6]	X[6]	
Endocrine function: fasting am cortisol, TSH	X	X[10]			X
Serum pregnancy test (If the patient is of child-bearing potential)	X[3]	X[3]			
Contrast enhanced MRI brain and/or spine; Body CT scans (if systemic disease present)	X		X[7, 12]	X	
MDASI-BT and/or MDASI-SP	X[9]		X**	X**	
Neurologic function score (See **16.3**)			X	X	
Adverse events evaluation		X[8]			

Calendar Key:
1 See section 2.3 for tests required and allowable time for screening. All others performed at baseline.
2 Within 14 days prior to treatment
3 Within 24 hours prior to treatment
4 It is acceptable to submit archived tumor that has been collected any time prior to registration into the study.
5 If lymphocyte count < 500 mm^3; If the CD4 is ≤ 200, then prophylaxis can be initiated at the discretion of the PI 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.
6 Blood samples (6 CPT tubes = 48 ml) will be collected within 14 days of treatment initiation, then prior to the administration of cycle 3, 5, 7, 13 and then at the tumor evaluation 2 months after completing therapy. If scheduling conflicts interfere with blood sample collection prior to odd cycles, samples can be obtained at the next even cycle (e.g., pre-C4). Patients stopping treatment early because of toxicity will have the final sampling done 2 months after completing treatment. At the NIH, these blood samples will be collected from the Clinical Center Phlebotomy Service by a member of the NOB Research Team, processed by centrifugation and then stored after barcoding in the NOB Laboratory at -80°C in a monitored freezer. Blood collection and processing contact (**NIH blood samples only**) is Dr. Wei Zhang office phone 240-760-6835 pager 102-12209 cell 240-274-0469. Please note that tubes and media may be substituted based on availability with the permission of the NCI PI or NCI laboratory investigator.
7 Imaging and MDASI questionnaires not necessary for Pre-Initiation of Cycle 1 as it was done for baseline (imaging if obtained within 28 days of C1D1; questionnaires if obtained within 14 days of C1D1).
8 See Section 7 for timelines for AE reporting.
9 MDASI-BT and/or MDASI-SP to be completed within 14 days prior to treatment and will be considered baseline
10 Tests done at screening or baseline do not need to be repeated at baseline or Day 1 of Cycle 1 if performed in designated time frame before start of study drug.
11 If the imaging schedule was altered due to a clinically indicated short interval scan, the history and physical may be rescheduled to coincide with the new imaging schedule.
12 If a short interval scan is clinically indicated, the imaging schedule may be changed to occur every 8 weeks post the short interval scan, per PI discretion (i.e., It will be done within 7 days of the initiation of even numbered cycles).
13 Individuals who do not meet the criteria for participation in this trial (screen failure) because of a reversible medical or surgical complication or a decision to proceed with another therapy may be rescreened once the underlying condition has resolved or they experience further tumor progression.

***** May use HBs Ag or HBV DNA Quantitative. Participants who are positive for hepatitis B core antibody, hepatitis B surface antigen (HBsAg), or hepatitis C antibody must have a negative polymerase chain reaction (PCR) prior to enrollment. Those who are PCR positive will be excluded. Participants with prior exposure to Hepatitis B virus but with natural immunity and no evidence of acute or chronic infection can be included, i.e.: AST and ALT within normal limits, Hep B core antibody total – positive, Hep B core antibody (IgM) – negative, Hep B surface antigen – negative, Hep B surface antibody – positive, Quantification of HBV PCR –negative. If HCV antibody is positive, obtain HCV RNA testing. If a false positive of HCV antibody is suspected (i.e., HCV antibody positive and HCV RNA testing negative), the Hepatitis C antibody test should be repeated, ideally in a different laboratory:

a. If NEGATIVE: allowed to proceed with Step 2 registration, as long as all other eligibility criteria are met.
b. If POSITIVE again: to proceed with Step 2 registration it is required to provide documentation of having discussed this result with the participant and ruling out recent or ongoing behavioral risks for HCV acquisition.

**** MDASI assessment** will take place along with imaging studies (every 2 cycles) but prior to the patient being informed of the imaging results. Patients off treatment because of disease progression will not undergo future imaging or PRO assessments on this protocol.

*** **Allowance for scheduling changes**: Brief interruption and delay in the 28-day cycle may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, insurance, and government holidays, etc. This can also extend to complications of disease not attributable to disease progression or protocol therapy. Therefore, for participants who have been on study therapy for 8 cycles or longer, the allotted window for delays will be extended to 6 weeks. For participants who have been on less than 8 cycles any interruption more than 14 calendar days will not be permitted. If nivolumab has been on hold for ≤ 14 days due to toxicity or other reasons, there is no need to repeat imaging before reinitiating treatment if imaging was already obtained at the beginning of the current cycle, unless deemed necessary in the opinion of the treating physician.

In this population of patients with rare CNS tumors, patients often start an alternative therapy after being taken off treatment or enter hospice care due to disease progression. As it is not always feasible to obtain the six assessments noted above, and at the designated times noted, every effort will be made to obtain the data. However, for this study, data is preferred but not required and will be obtained per investigator discretion.

3.7 COST AND COMPENSATION

3.7.1 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 an insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

For NCI-CONNECT Participating Site:

Participants' costs will be based on local guidelines as described in the site specific consent.

3.7.2 Compensation

Participants will not be compensated on this study.

3.7.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation for participants seen at the NIH Clinical Center. 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.

For NCI-CONNECT Participating Site:

Participants reimbursements will be based on local guidelines as described in the site-specific consent.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.8.1 Criteria for removal from protocol therapy

Prior to documenting removal from protocol therapy, effort must be made to have all participants complete a safety visit approximately 30 days following the last dose of study therapy.

NOTE: The collection of toxicities should continue until at least 100 days as outlined in section **6.1.1**. The 100-day evaluations may be collected remotely (e.g., phone or an institutionally approved teleconference platform) at the discretion of the investigator without requiring an in-person visit or any other tests/procedures.

In the absence of treatment delays due to adverse event(s), treatment may continue as specified in the above treatment modality sections or until one of the following criteria applies:

- Disease progression.
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s) as described in Section **3.3**
- Participant decides to withdraw consent for active treatment in the study
- General or specific changes in the patient's condition render the participant unacceptable for further treatment in the judgment of the investigator.
- Participant has received the maximum number of doses allowed*
- Positive pregnancy test

***Note about the maximum number of doses allowed:**

- If a participant misses a dose due to non-AE related issues, the dose can be given later.
- If a dose is held due to concern about an AE, it may be given at a later time if it is determined that the issue was NOT related to treatment.
- In the case of a verified treatment-related AE that does NOT result in requiring the participant go off treatment, reinstitution of treatment may be considered at the discretion of the PI. Participants taken off study treatment because of treatment toxicity are not allowed to re-initiate treatment.

3.8.2 Treatment Beyond Imaging Determination of Disease Progression

Accumulating evidence indicates that some subjects treated with immune system stimulating agents may develop apparent progression of disease (by conventional response criteria) before demonstrating clinical objective responses and/or stable disease. This phenomenon was observed in approximately 10% of subjects in the Phase 1 study of nivolumab in patients with glioblastoma.

In order to minimize premature discontinuation of study medication and distinguish pseudoprogression from progressive disease, participants initially meeting radiologic criteria for disease progression may continue receiving study medication until confirmation of progression with an MRI performed within 12 weeks. This criterion would apply anytime during the course of treatment with the experimental agent, given that there are no established guidelines for the timing of checkpoint-inhibitor-related pseudoprogression in the primary CNS patient population. In order to continue study treatment after assessment of initial radiological progression, the following criteria must be met:

1) The participant is believed to demonstrate clinical benefit as determined by the investigator,

2) The participant is tolerating study medication,

3) There has not been a significant worsening of neurologic function or decline in performance status. However, participants with clinical worsening who respond to corticosteroids as outlined in the iRANO criteria or who undergo sampling of the enlarging mass that does not demonstrate tumor progression can remain on or resume treatment.

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

In those cases, in which radiologic progression cannot be differentiated from pseudoprogression and it is the investigator's opinion that a surgical biopsy or resection to obtain tumor tissue for histopathology is in the participant's best interests, a surgical resection may be performed following consultation with the Principal Investigator. Tumor biopsy samples (tissue or slides) must be submitted for central review by a neuropathologist to minimize any inter-observer variation in the histopathologic assessment of progression versus treatment-related changes. If tumor pathology confirms progression, then the participant will be discontinued from study medication per protocol discontinuation criteria and the date of progression will be the day that it was first suspected. If tumor pathology reveals treatment-related changes and does not confirm disease progression, a discussion with the Lead NCI study PI is required to potentially allow the

participant to continue study medication. An MRI after the resection is required prior to treatment continuation. The participant will then continue all on-treatment tumor assessments as per the treatment schedule.

3.8.3 Off-Study Criteria

The following criteria will be used to take participants off study (no further data can be collected)

- Completed study follow-up period
- Screen Failure
- Investigator discretion
- Participant requests to be withdrawn from study
- Death
- Lost to follow up
- Study is cancelled for any reason

3.8.4 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 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 within 24 hours and reschedule the missed visit within 7 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

4.1 PERMITTED SUPPORTIVE/ANCILLARY CARE AND CONCOMITANT MEDICATIONS

All supportive therapy for optimal medical care will be given during the study period at the discretion of the treating physician(s) within the parameters of the protocol and documented on each site's source documents as concomitant medication.

- *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 Section **16.4** 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 Jirovecii Prophylaxis*: Patients with severe lymphopenia are at an increased risk for opportunistic infections. Participants 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 Section **16.4**. Diarrhea is a frequent toxicity associated with nivolumab so strict adherence to the guidelines is required.

4.2 PROHIBITED THERAPIES

- No other investigational drugs will be allowed.
- Surgical procedures for tumor debulking, other types of chemotherapy, and immunotherapy or biologic therapy must not be used. Stereotactic boost radiotherapy or other forms of local treatment such as the Gliadel wafer are not allowed.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 BIOSPECIMEN COLLECTION (SEE 16.6)

Note: Platforms and procedures for analysis may be adjusted based upon current technology and/or collaborations in place at the time of actual analyses.

5.1.1 Tumor Tissue

Tumor tissue (Formalin-fixed paraffin embedded material) will be collected on all participants prior to Step 2 registration. Tumor blocks or unstained slides will be utilized to generate genomic material for molecular profiling of the tumor cells and the tumor microenvironment including the immune-related cells. When available, fresh, or fresh-frozen tumor material will be collected for these studies as well as characterization by flow cytometry.

Immune Phenotyping

We hypothesize that the tumor microenvironment of participants who will respond to immune therapy contains both cells with cytolytic capacity as well as cells, including tumor, that express immune regulatory molecules. We further hypothesize that with checkpoint blockade treatment, the microenvironment will be tipped in favor of anti-tumor immunity. Checkpoint blockade has

been shown to be beneficial in cancers that possess this immune profile of opposing cytotoxic and immune suppressive molecules, including melanoma and colorectal cancers with genetic instability (69, 70).

To profile the tumor microenvironment, fresh tumor samples will be analyzed by comprehensive flow cytometry panels (detailed information in Appendices section **16.6**). Our current analyses of glioma brain tumor samples demonstrate high levels of CD8 T cells relative to CD4 T cells. These lymphocytes express markers associated with activation and exhaustion, including CXCR3, ICOS, PD-1, CTLA-4, TIM-3, and LAG-3. Consistent with this, Rooney, et al. demonstrated statistically significant increases in transcript signatures of cytolytic activity in both glioma and glioblastoma samples relative to normal brain (p=0.049 and p=1.1x10^{-37}, respectively) (71). These data demonstrate that an activated microenvironment is present in brain tumors, and we can assess the immune profile using flow cytometry.

Tumor blocks or slides will be assessed for immune profiles using quantitative immunofluorescence. Immune subtype frequency, spatial location of these subtypes in the TME with respect to each other and the tumor mass, and presence of activation markers will be assessed by quantitative microscopy. Immunologic parameters quantified by microscopy will be validated by flow cytometric analysis when fresh tumor tissue is available. These correlates of immune response will have broad application to immune therapy clinical trials.

Because few tumor cells are retained in samples for flow cytometry, the primary tumor will be assessed for immune regulatory molecules by quantitative immunofluorescence. Because PD-L1 binds to PD-1 and provides a negative signal to T cells, PD-L1 levels will be quantified in the tumor microenvironment. As described above, rare tumors, such as malignant meningioma, express exceptionally high levels of this inhibitory molecule. In addition, PD-L1 expression increases with biologically relevant stimuli such as hypoxia and interferon gamma (see data above). PD-L1 expression on hematopoietic cells will be quantified by flow cytometry and immunofluorescence.

5.1.2 Blood

Blood specimens will be obtained for the purpose of determining immunocompetance of T-cell and NK cell function. Blood samples will be drawn prior to the initiation of treatment of cycle 1, then prior to the administration of cycle 3, 5, 7, 13 and then at the tumor evaluation 2 months after completing therapy. Participants stopping treatment early because of toxicity will have the final sampling done 2 months after completing treatment. A total of 60 cc of blood will be taken for analysis at each time point.

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 microL) has been found to a negative marker of response to anti-CTLA-4 (61, 72) 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 (73) and IL-2 therapy for melanoma (74) (75).

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 participant 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 treatments. (76, 77)

The immune phenotype of peripheral blood will be analyzed by flow cytometry both as a reference for lymphocyte gating and to assess its correlative potential with the primary tumor in determining response to checkpoint blockade. We hypothesize that the primary tumor microenvironment will be enriched for tumor-reactive lymphocytes, however, these lymphocytes may also be present in peripheral blood. Gros, et al identified tumor-reactive T cells in the PD-1^{high} population of peripheral blood. (78) The T cell receptor specificity and repertoire of these circulating T cells were similar to those found in the tumor microenvironment.

The immune profile of longitudinal peripheral blood samples and, if available, tumor resected due to progression/pseudoprogression will be assessed to detect and correlate changes in immune profile with outcome. Complete blood counts with differential will be determined on each blood draw to compare the percent and absolute count of hematopoietic subsets.

Correlates of tumor reactivity

Correlates with tumor reactivity will be assessed retrospectively in participants who respond to checkpoint blockade determined by progression-free survival. T cell reactivity to tumor antigens will be assessed by response to peptide presentation on autologous antigen presenting cells (APCs). To maximize leukocyte response, we will immortalize donor-derived B cells to serve as APCs. Activated B cells express high levels of MHC class I and II molecules as well as CD80 and CD86 co-stimulatory molecules, thereby making these cells potent APCs (79). Tumor antigens will be introduced by electroporating the B cells with either a tumor RNA library as an unbiased approach or with a tandem minigene library. T cell response will be assessed by flow cytometric evaluation of cytokine production as well as interferon gamma ELISpot to confirm and quantify reactive lymphocytes. The T cell repertoire over time will be monitored by genetic analysis of longitudinal samples, as successful checkpoint blockade therapy has been shown to cause an expansion in this repertoire.

Identification of tumor antigens

For selected cases, determined based on extraordinary response or atypical activity, genomic analyses will be performed to identify tumor antigens. Whole exome sequencing and

transcriptome analysis of the tumor will be used to identify tumor associated antigens and neoantigens. In addition, the specific HLA expressed by the tumor will be determined. The list of tumor antigens will be narrowed based on those that are efficiently processed by the proteasome and are expressed by the HLA on the tumor. These sequences will be used to test PBMC reactivity to tumor antigens.

Finally, 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 (polymorphisms) to search for predictors of response and/or nivolumab toxicity.

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Slides and tissue blocks will be stored in the NCI Laboratory of Pathology and/or SNB Tissue Bank (if surgery performed at the NIH) for potential future studies. The SNB Tissue Bank is located in Building 10. Paraffin embedded formalin fixed tissues and slides will be stored in locked cabinets and accessible only to authorized personnel (the investigators and technicians involved in this study). Frozen materials (tumor, blood, or blood components) will be stored in locked, monitored-80°C freezers. Data will remain in locked filing cabinets and/or on password-protected computer servers in the SNB offices and/or NCI designated system such as Labmatrix, which is accessible only to authorized personnel (the investigators and technicians involved in this study). Tissue will be labeled only by a participant identifier code and will be catalogued and stored in the Laboratory of Pathology and/or SNB tissue banks. Blood samples will also be labeled only by a participant identifier and stored in the NCI lab.

5.2.1 NCI Lead Site

Blood 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.

All samples sent to the Neuro-Oncology, Laboratory of Pathology, or the Brain Tumor Tissue Bank (housed in the SNB) will be barcoded with data entered and stored in the Labmatrix system utilized by the CCR, NCI. This is a secure system with access limited to defined personnel.

Labmatrix creates a unique barcode ID for every sample which cannot be traced back to patients without Labmatrix access. Data will be recorded as applicable for each sample (e.g., patient ID, name, trial name/protocol number, date/time drawn, as well as box and freezer location). Participant demographics associated with the Clinical Center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

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

If the participant withdraws consent the participant's 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**.

5.3 MULTI-SITE INSTRUCTIONS FOR TISSUE COLLECTION AND SHIPMENT

5.3.1 Central Pathology Tissue Submission for Molecular Analysis (Step 1 Registration)

Central pathology tissue submission is **mandatory** for this study and tissue submission must occur in conjunction with Step 1 Registration. Tumor tissue from non-NIH participating sites will be batched for analysis. The NCI Laboratory of Pathology will perform a pathology review for every case. The pathology review will consist of: (1) confirmation that the histologic features meet the WHO criteria for one of the rare CNS tumors; and (2) confirmation that the tissue is of sufficient size for determination of molecular profile (MCP) and immunology profile.

Tissue specimens should be taken from pre-study diagnostic open biopsy or surgical resection (ideally from the most recent recurrence if possible).

The following materials are required:

Representative tissue blocks that contain diagnostic viable tumor.

As a guide, sufficient tumor tissue should be present to generate a 1 x 1 cm square area of tumor when the block is cut onto a slide. Note that the tissue blocks composed primarily of either normal tissue or necrotic tissue are inadequate for molecular analysis, as it depends on the presence of viable tumor tissue. In cases where a single block has insufficient tumor, tissue for multiple blocks can be combined to ensure specimen adequacy. If the NCI Laboratory of Pathology determines that the block that was sent is insufficient, the NCI-CONNECT Study Coordinator/Research Nurse will contact the site in an attempt to obtain additional tissue. *Submission of at least 2 blocks is highly encouraged*. One or both blocks will be returned upon request.

A Pathology Report documenting that the submitted material contains tumor; the report must include the Nivo Rare CNS Tumor protocol number and the patient's initials. The patient's name and/or other identifying information will be removed from the report. The surgical pathology numbers and information must NOT be removed from the report.

A Specimen Transmittal Form (STF) listing pathology material being submitted for Central Tissue Evaluation completed by the local pathologist must be included in the pathology submission. These forms must include the Nivo Rare CNS protocol number and the patient's initials. An accompanying H&E is strongly encouraged.

Send pathology material by overnight mail directly to:

NCI-CONNECT Coordinating Center
Neuro-Oncology Branch
National Cancer Institute
9030 Old Georgetown Road
Building 82, Room 229
Bethesda, MD 20892
240-760-6060

5.3.2 Tissue Shipments Instructions

Shipments must be made Monday through Thursday.

Notify the NCI-CONNECT clinical research nurse coordinator by emailing NCI17C0102TissueLogistics@mail.nih.gov on or before the day of submission with the following information: (1) that a case is being submitted (2) the name of the contact person; (3) when to expect the sample; and (4) the overnight shipping carrier and tracking number.

Tumor tissue review by NCI Laboratory of Pathology will NOT need to be completed before Step 2 registration.

However, if the outside pathology report does not provide an unequivocal diagnosis, central NIH review is mandatory before Step 2 registration. The NCI-CONNECT Coordinating Center will then notify the appropriate contact person from the submitting institution with the results.

If the participant does not meet eligibility requirements, *all* tissue blocks and forms will be returned to the participating submitting institution.

The NCI Laboratory of Pathology will cut sections for DNA/RNA isolation and perform the molecular profile. The immunology profile analysis will be performed at the Neuro-oncology Branch (NOB) Laboratory.

NOTE: Samples must be shipped to the NCI Laboratory of Pathology during the weekdays (**Monday-Thursday morning**) by overnight priority shipping/courier at room/ambient temperature (20-30°C). Please review the Laboratory Manual for additional details on tissue submission.

5.3.3 Immune Monitoring Blood collection:

See Central Laboratory Manual for collection, preparation, and shipment instructions of correlative blood samples. The Central Laboratory Manual will be distributed to sites by the coordinating center.

5.4 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.4.1 Description of the scope of genetic/genomic analysis

Tumor and blood specimens will be collected, and genomic analyses performed on each. 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 will be obtained from white blood cells from peripheral blood. Whole exome sequencing will be performed for the neoantigen analysis described previously and evaluation of single nucleotide polymorphisms will be obtained for evaluation of SNPs that predict for immunologic response or treatment-related toxicity.

5.4.2 Management of Results

The analyses that we perform in our NCI laboratory are for research purposes only; they are not nearly as sensitive as the tests that are performed in a laboratory that is certified to perform genetic testing. Changes that we observe unrelated to our research may or may not be valid. Therefore, we do not plan to inform participants of the results of testing on the tissue and blood

that is performed in our research lab. However, we plan to contact participants if a clinically actionable gene variant sought during the analysis performed in the Neuro-oncology Research Laboratory is discovered. The planned analysis will be performed as batched analysis at the conclusion of the study enrollment and all participants have completed study procedures. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of secondary findings that is current at the time of primary analysis.

Unless otherwise requested, participants will be contacted at this time with a request to provide a sample to be sent to a CLIA certified laboratory for verification. **For participants enrolled at the NIH,** the CLIA testing may be funded by the PI or the CCR. Once verified, the participant will be offered the opportunity to schedule a visit, in person or via telemedicine on an NIH approved platform to have genetic education and counseling to explain this result. If the participant does not want to come to NIH or participate in a telemedicine visit, a referral to local genetic healthcare provider will be provided (at their expense).

For participants enrolled at non-NIH sites, the reviewing IRB will determine whether or not incidental findings are returned to the participants. In these cases, the costs of confirmatory CLIA testing would not be covered by the PI or the CCR. If findings are verified in a CLIA lab, the participant will be referred to a local genetics' health care provider.

This is the only time during the course of the study that secondary findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

Participants will be requested to maintain up to date contact information to enable future contact.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Data will be collected using a data management system created in a 21 CRF Part 11 compliant database developed by NCI CCR. Participants will enter their responses to the MDASI-BT and/or MDASI-SP questionnaires directly in Scribe, and the MDASI-BT and MDASI-SP data will be stored in Labmatrix. Labmatrix will remain part of the database management system.

Participating sites will be trained on the electronic data capture (EDC) system and Scribe, and NIH personnel will also be trained on Labmatrix. This system will allow acquisition of clinical data, imaging results, laboratory studies comprising both the clinical monitoring studies such as the CBC, chemistries etc. as well as the results from the correlative biologic assessment of immune competency and the tumor immunophenotype.

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11 compliant data capture system provided by 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, Study Day 1, through 100 days after the study agent was last administered. Adverse events that are serious need to be recorded through 100 days. Beyond 100 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

6.1.1 Collection of Adverse Events

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

Related: There is a reasonable causal relationship between study drug administration and the AE.

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

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

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

The collection of nonserious AE information should begin at initiation of study drug. All nonserious 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.

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

An abnormal laboratory value will be considered an AE 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 participant’s outcome.

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

6.1.2 Source Documentation Timeframes

The following information will be entered into the electronic data capture system (EDC) and Scribe/Labmatrix within the indicated timeframes. In addition, the source documents should be provided to the NCI-CONNECT Coordinating Center's Research Nurse by encrypted email, or Secure Email and File Transfer (SEFT) within the indicated timeframe. Participants will enter their responses to the MDASI-BT and/or MDASI-SP questionnaire directly in Scribe. Therefore, there will be no separate source document.

Data Set / Source Documents	Schedule for Submission
Regulatory Documents (as described on the BTTC/NCI-CONNECT wiki site https://ccrod.cancer.gov/confluence/display/BTTC/Home)	Prior to Step 1 of registration
Registration Form (eligibility checklist)	Within 24 hours of signing Informed Consent (**Step 1 registration**) **For outside non-NIH sites only**: Following confirmation of eligible diagnosis based on outside pathology report, or confirmation of adequate tissue by NCI Laboratory of Pathology if the outside pathology report is not conclusive (**Step 2 registration**)
Copy of signed & dated Informed Consent w/ HIPAA Authorization	Within 24 hours of signing Informed Consent (Step 1 registration)
Pathology Report (from the most recent pre-registration diagnostic biopsy or surgery)	With submission of tissue (between Step 1 and Step 2 registration)
Baseline MDASI – BT and/or MDASI-SP Questionnaire	Within 14 days prior to treatment
Screening Source Documents	At time of registration (Step 2)
Other Source Documents	With Participant Status Updates Form

6.1.3 Database Entry Timeframes

The following data should be entered into the electronic data capture system (EDC) and Scribe/Labmatrix within the specified timeframes. Source documentation will be kept at the participating site. Sites (NCI and NCI-CONNECT participating sites) will be responsible for entering data collected at their site.

Data Set	Schedule for Data Entry
Screening data	Within 14 days of Step 1 Registration
Baseline data	No later than 14 days after Day 1 of cycle 1 drug administration
Treatment (Course/Cycle) Data (To include treatment, response, AE, and clinical evaluation information)	Course initiation case report form (CRF) no later than 7 days after the cycle begins All other course/cycle CRFs must be entered no later than 21 days after the cycle is complete
Off Treatment Data	Within 21 days after the: • last date of any modality of protocol treatment; or, • study discontinuation visit.
Follow-up (Survival) Data (To include post-treatment medication and long-term follow up survival data) Note: Long-term follow up is required until patient is off study.	No later than 21 days after follow-up visit or survival call or post-treatment medication begins.
Non-Treatment Data (May include Patient Reported Outcomes questionnaires (MDASI-BT and/or MDASI-SP), Specimen Tracking information, Pathology Specimen Submission, etc.)	Within 14 days after each scheduled assessment, event, or activity
Off Study Data	No later than 21 days after the date the patient is removed from the study.

6.1.4 Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Any source documents submitted to the NCI-CONNECT Coordinating Center with protected health information or personally identifiable information (e.g., participant's name) must be sent via Secure Email and File Transfer (SEFT) or secure fax. For NIH only, documents can also be sent via hand-delivery or encrypted email.

6.1.5 Safety Data

All participants receiving study agents will be evaluated for safety. The safety parameters include all safety laboratory tests listed in the Study Calendar, CNS observations, physical examination findings, and spontaneous reports of adverse events reported to the investigator by patients. Life-threatening toxicities that are unexpected and assessed to be possibly related to the study agent should be reported immediately.

Information about all adverse events, whether volunteered by the participant, discovered by investigator questioning, or detected through physical examination, laboratory test or other

means, will be collected and recorded on the Adverse Event Case Report Form and followed as appropriate.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment (any procedures specified in the protocol). Adverse events occurring before starting study treatment but after signing the informed consent form are recorded on the Baseline Evaluations Case Report Form.

6.1.6 Patient-reported outcomes

Received MDASI-BT and MDASI-SP questionnaires will be checked versus the timing schedule and considered as valid if they fall within 14 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 participants 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 participants rating their symptoms to be 5 or greater (on a 0-10 scale) will also be reported. We will construct individual participant profiles for each of the selected symptoms to describe the individual participants' 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 participants with at least one valid questionnaire will be included in the analyses. Questionnaires completed at study registration will be considered baseline.

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.

6.2 HUMAN DATA SHARING, INCLUDING GENOMIC DATA SHARING, AND PUBLICATION

6.2.1 NIH Data Management and Sharing Policy and NIH Genomic Data Sharing Policy Compliance

This study will comply with the NIH Data Management and Sharing (DMS) Policy, which applies to all new and ongoing NIH-funded research in the IRP, as of January 25, 2023, that is associated with a ZIA, with a clinical protocol that undergoes scientific review and/or will involve genomic data sharing.

This study will comply with the NIH Genomic Data Sharing (GDS) Policy, which applies to all new and ongoing NIH IRP-funded research, as of August 31, 2015, that generates large-scale human or non-human genomic data, as well as the use of these data for subsequent research. Large-scale data include genome-wide association studies (GWAS), single nucleotide polymorphisms (SNP) arrays, and genome sequence, transcriptomic, epigenomic, and gene expression data.

The assessments performed on this study do not meet thresholds/criteria for the NIH GDS Policy.

6.2.2 NIH Public Access Policy Compliance

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

6.3 RESPONSE CRITERIA

This study will utilize the newly developed iRANO (Immunotherapy Response Assessment Neuro-Oncology) assessment criteria that provides special consideration for the development of pseudoprogression, 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 **16.5.**

Given that some participants with the selected rare CNS tumors eligible for this trial may have systemic tumor involvement, immune RECIST (iRECIST) will be used to evaluate response in extra CNS lesions (see Appendix **16.5**) (80).

6.3.1 Definitions

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

Evaluable for objective response: Only those participants 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 objective response. These participants will have their response classified according to the definitions stated below. However, all participants regardless of the presence or absence of measurable disease will be evaluated for the co-primary endpoint of 6 month progression free survival. (**Note**: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable for the primary endpoint of disease control rate (DCR) are all participants who were fully enrolled to the trial (i.e., completed Step 2 registration) and received at least 1 full cycle of nivolumab (2 doses). Participants with disease progression or major drug toxicity preventing them from continuation of protocol therapy prior to the end of cycle 1 will also be considered evaluable. Major drug toxicity is defined as drug toxicities leading to drug discontinuation with respect to evaluable participants.

6.3.2 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation. For non-measurable disease, assessments will be categorized as stable, improved, or worse. All baseline evaluations must be performed before beginning of treatment.

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

6.3.3 Response Criteria: RANO Criteria with the iRANO update (81) (82) (Summarized in the Table 2 below)

6.3.3.1 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.
- Participants 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

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

6.3.3.2 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.

6.3.3.3 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 participants 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 participant experiences significant clinical decline at any time, the date of actual progression will be back-dated to the first date that the participant met criteria for progression and such patients should discontinue further immunotherapy.

6.3.3.4 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.

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

The modified RANO Response Criteria with the recent iRANO modifications to be used for immunotherapy agents are summarized in this table.

Table 2 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

Participants 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, participants 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.

6.3.4 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.

6.3.5 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 pseudoprogression, participants with worsening lesions but without significant clinical decline may continue on treatment. Subsequent confirmation of disease progression will mandate that the date of progression be defined as the date imaging worsening was first discovered. Participants who undergo diagnostic biopsy or resection uncovering evidence of pseudoprogression will NOT be declared as having disease progression and will be able to resume protocol treatment if considered appropriate after discussion with Lead NCI study PI.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant 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.htm).

7 NIH REPORTING REQUIREMENTS/DATA SAFETY MONITORING PLAN (NIH SITE ONLY)

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at https://irbo.nih.gov/hrpp-policy-guidelines/.

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/hrpp-policy-guidelines/.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at https://irbo.nih.gov/hrpp-policy-guidelines/.

7.3 NCI Clinical Director Reporting

This only applies to the NIH site.

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 **s**hould 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 NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NCI Guidance For Expedited Reporting For Multi-Center Trials

Report events to the Reviewing IRB as per its policy. Please also notify the NCI-CONNECT Coordinating Center Lead PI and Research Nurse Coordinator of your submission at the time you make it.

For IND studies, the site PI will directly submit reports to the CCR as IND sponsor per section **8.3.**

Events will be submitted by participating sites directly to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov per section **8.3** and must also email the NCI-CONNECT Lead PI and Research Nurse Coordinator.

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 NCI Lead Principal Investigator/Research Team

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

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 events and response data on each participant 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 **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 the time of 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 requires 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 **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 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATOR (BMS)

8.4.1 To Be Sent by OSRO Safety to Bristol Myers Squibb (BMS)

8.4.1.1 Serious Adverse Event Collection and Reporting

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 within 100 days of discontinuation of dosing.

All SAEs must be collected that occur during the screening period. If applicable, SAEs must be collected that relate to any protocol-specified procedure (e.g., a follow-up skin biopsy). 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. SAEs must be recorded on BMS or an approved form; pregnancies on a MedWatch Form.

8.4.2 To Be Sent by NCI Study Personnel to BMS

8.4.2.1 Nonserious Adverse Event Collection and Reporting

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

The collection of nonserious AE information should begin at initiation of study drug. All nonserious 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.

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

Bristol-Myers Squibb reporting contact information:

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

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

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

All SAEs should be followed to resolution or stabilization.

8.5 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

8.5.1 Maternal exposure

If a participant 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 from 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 becomes known,

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

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

8.6 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.

8.7 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.

Non-NIH participating sites not using the CCR Protocol Deviation Tracking System (PDTS) will report any protocol deviation on the OSRO Site Protocol Non-Adherence/Deviation Log, or a site-generated protocol deviation report approved by OSRO. The Non-Adherence/Deviation

Log should be maintained in the site essential documents file. **By the end of each month for the duration of the trial**, non-NIH sites will submit the log to the NCI-CONNECT Research Nurse Coordinator at nci_bttc@mail.nih.gov and the NCI-CONNECT Research Nurse will submit the cumulative log to OSRO via OSROMonitoring@mail.NIH.gov on the **first business day of each month throughout the study**.

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

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 DESIGN

As described in greater detail below, participants will be assigned to cohorts based on the number of prior therapies (radiation and/or standard or investigational antineoplastic drugs). There will be 2 cohorts: heavily pretreated and non-heavily pretreated. A total sample size of 75 participants is planned for each of the two cohorts. Accrual will be limited to 75 participants per cohort, but within a particular cohort, any of the disease types can be accrued without quotas until the limit is reached.

The accrual is up to 150 evaluable study participants who completed Step 2 registration and undergo at least 1 cycle (2 infusions of nivolumab). Accounting for 20% of screen failures occurring between Step 1 and Step 2 or inevaluable participants (i.e., those who signed Step 2 but did not complete Cycle 1 for reasons other than toxicity or PD), overall enrollment is 180 study participants. We anticipate 22 screen failures and 8 inevaluables. Therefore, we estimate that up to 158 participants will start study drug: up to 89 at the NIH and up to 69 participants will start study drug at non-NIH participating sites.

Evaluable subjects for the primary endpoint of DCR (disease control rate) are all participants who were fully enrolled to the trial (i.e., completed Step 2 registration) and received at least 1 full cycle of nivolumab (2 doses). Participants with disease progression or major drug toxicity preventing them from continuation of protocol therapy prior to the end of cycle 1 will also be considered evaluable. Major drug toxicity is defined as drug toxicities leading to drug discontinuation with respect to evaluable participants.

The primary endpoint is the disease control rate (DCR), which is defined as the rate of achieving a confirmed CR/PR (confirmation by imaging at least one month later) or durable SD lasting at least 6 months (PFS-6). A maximum sample size of 75 participants is planned for each of the two cohorts, with an interim analysis planned when the sample size reaches 32. We monitor the DCR of the heavily pretreated cohort and the non-heavily pretreated cohort independently using the Bayesian optimal phase 2 (BOP2) design (83). Specifically, let n denote the interim sample size and N denote the maximum sample size. Let p_{DC} denote the true DCR.

For the heavily pretreated cohort, define the null hypothesis H_0:$p_{DC} \leq 0.1$, representing that the treatment is inefficacious. We will stop enrolling participants and claim that the treatment is not promising if

$$Pr(p_{DC} > 0.1|data) < \lambda(\frac{n}{N})^{\alpha},$$

where λ=0.9 and α=1 are design parameters optimized to maximize power under the alternative hypothesis H_1:$p_{DC} = 0.2$, while controlling the type I error rate at 0.1 (i.e., the chance of incorrectly claiming that an inefficacious treatment is promising is no more than 10%). Assuming a Beta (0.1,0.9) prior distribution for p_{DC}, the above decision rule corresponds to the following stopping boundaries and yields a statistical power of 0.8324 under H_1:

Table 3: Optimized stopping boundaries for heavily pretreated cohort

# patients treated	Stop if # disease controls <=
32	2
75	11

Based on Table 3, we perform the interim analysis for the heavily pretreated cohort when the number of enrolled participants reaches 32. When the total number of participants reaches the maximum sample size of 75, we reject the null hypothesis and conclude that the treatment is promising if the number of participants with disease control are greater than 11; otherwise, we conclude that the treatment is not promising. Below are the operating characteristics of the design based on 10000 simulations using the BOP2 web application, which is available at http://www.trialdesign.org.

Table 4: Operating characteristics for heavily pretreated cohort

True DCR	Early stopping (%)	Claim promising (%)	Sample size
0.10	36.83	6.48	59.2
0.15	12.26	44.13	69.7
0.20	3.41	83.24	73.5
0.30	0.14	99.71	74.9

For the non-heavily pre-treated participants, define the null hypothesis H_0:$p_{DC} \leq 0.2$, representing that the treatment is inefficacious. We will stop enrolling participants and claim that the treatment is not promising if

$$Pr(p_{DC} > 0.2|data) < \lambda(\frac{n}{N})^{\alpha},$$

where λ=0.875 and α=1 are design parameters optimized to maximize power under the alternative hypothesis H_1:$p_{DC} = 0.3$, while controlling the type I error rate at 0.1 (i.e., the chance of incorrectly claiming that an inefficacious treatment is promising is no more than 10%). Assuming a Beta (0.2,0.8) prior distribution for p_{DC}, the above decision rule corresponds to the following stopping boundaries and yields a statistical power of 0.7569 under H_1:

Table 5: Optimized stopping boundaries for non-heavily pretreated cohort

# patients treated	Stop if # disease controls <=
32	5
75	19

Based on Table 5, we perform the interim analysis for the non-heavily pretreated cohort when the number of enrolled participants reaches 32. When the total number of participants reaches the maximum sample size of 75, we reject the null hypothesis and conclude that the treatment is

promising if the number of participants with disease control are greater than 19; otherwise, we conclude that the treatment is not promising. Below are the operating characteristics of the design based on 10000 simulations using the BOP2 web application, which is available at http://www.trialdesign.org.

Table 6: Operating characteristics for non-heavily pretreated cohort

True DCR	Early stopping (%)	Claim promising (%)	Sample size
0.1	90.30	0.00	36.2
0.2	36.16	9.30	59.5
0.3	5.26	75.69	72.7
0.4	0.34	99.22	74.9

During the interim analysis, if the go/no-go decision cannot be made because of pending participants whose disease control status has not been cleared, the method of Cai et al. (84) may be used to impute the outcome for the pending participants to facilitate real-time decision making when needed.

10.1.1 Stopping rule for treatment-related deaths

If ≥1 treatment-related death occurs in any of the 2 cohorts, then further accrual to the study will be suspended until a full review of safety is performed with the study sponsor.

10.2 Statistical Analysis Plan

10.2.1 Analysis of the Primary Endpoints

Point estimate and its standard deviation and 95% confidence interval will be calculated for the objective response and 6-month PFS for the non-heavily pre-treated cohort and the heavily pre-treated cohort, respectively. Kaplan-Meier estimates will be used to describe the PFS for each cohort.

10.2.2 Analysis of the Secondary Endpoint(s)

Kaplan-Meier estimates will be calculated for the OS for each cohort. Summary statistics, including mean, standard deviation and 95% confidence interval will be calculated for symptom burden (e.g., MDASI-BT or MDASI-SP module) at each time point for each cohort. One-sample t-test will be used to compare the symptom burden between different time points. Two-sample t-test will be used to compare the symptom burden between responders and non-responders. Linear mixed model will be further used to assess the trajectory of the longitudinal measures of symptom burden and compare responders and non-responders.

10.2.3 Safety Analyses

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

10.2.4 Exploratory Analyses

Logistic regression will be carried out to investigate the relationship between pretreatment immunocompetence defined by peripheral blood mononuclear cell assays and the response to

nivolumab. Proportional hazard model will be used to evaluate its relationship with the PFS and OS. Similar analysis method will be used to evaluate the relationship between a change in immunocompetence over the course of the study treatment and response and/or treatment-related toxicity.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

The investigational study agent is provided by Bristol-Myers Squibb under a Clinical Trials Agreement (CTA # 01039-17). Results or study data may be communicated to CTA partner Bristol-Myers Squibb according to the terms of the NIH- Advanced Accelerator Applications SA CTA.

11.2 MULTI-INSTITUTIONAL GUIDELINES

11.2.1 Participating Institution Site Initiation

Before an institution may begin participating in an NCI-CONNECT protocol, they must complete the following steps:

- Submit all required regulatory documents to the NCI-CONNECT Coordinating Center
- Participate in a site initiation visit, webcast, or conference call
- Receive training regarding study specific CRF's and/or databases
- Additional steps required for site activation will be provided by the NCI-CONNECT Coordinating Center

After these requirements have been fulfilled, the participating institution will receive by fax, e-mail, or hard copy, a Site Activation Memo. Once the Site Activation Memo has been received, the participating institution may begin to register participants to the protocol.

Documents requiring submission to the Site's reviewing IRB, including local consent documents generated from an approved model consent, should be provided to the coordinating center for central review prior to the IRB submission.

11.2.2 IRB Approvals

The Lead PI will provide the NIH Intramural IRB with a copy of the participating institution's initial approval and approved yearly continuing review. Registration will be halted at any participating institution in which a current continuing approval is not on file at the NIH Intramural IRB.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR PARTICIPANT SELECTION

Adult patients with selected recurrent, refractory rare primary central nervous system tumors will be invited to enroll onto this trial provided they meet the eligibility criteria outlined in Section **2.1.** No sex, racial or ethnic groups will be excluded from participation in this trial. Some of the participants could become cognitively impaired as a consequence of their tumor.

12.2 PARTICIPATION OF CHILDREN

Children (<18 years) will not be included in this protocol due to limited data on Nivolumab in children and the differing biology of childhood malignancies.

12.3 PARTICIPATION OF ADULTS UNABLE TO GIVE CONSENT (NCI NOB ONLY)

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that participants enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. This would not apply for reasons for inability to provide consent that are related to tumor progression as this would mandate that the participant stop treatment on the protocol. However, because in other circumstances, there is a prospect of direct benefit from research participation (Section **12.5**), 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) to assess ongoing capacity of the subjects and to identify an LAR, as needed. Please refer to section **12.7.1** for consent procedure.

12.4 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT (NCI-CONNECT PARTICIPATING SITES)

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that participants enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. This would not apply for reasons for inability to provide consent that are related to tumor progression as this would mandate that the participant stop treatment on the protocol.

Each institution will follow their own local policies and procedures for assessing capacity for re-consenting participants unable to re-consent and will notify the NCI-CONNECT Coordinating Center at the time of continuing review of these occurrences.

12.5 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

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 rare CNS tumors. The protocol provides for detailed and careful monitoring of all participants to assess for toxicity. Toxicity data from the current dose level will be collected and reviewed to ensure that there were no severe toxicities that would preclude further participant enrollment. Participants will be treated with therapeutic intent and response to the therapy will be closely monitored.

Additional risks are also those associated with the care performed at the NIH Clinical Center such as MRI and blood draws. In addition, questionnaires may contain questions that are sensitive in nature.

This study may involve unpredictable risks to the participants.

Benefits of participating in this study are that patients may receive expert neuro-oncologic opinion regarding their treatment. Many community providers are unfamiliar with management of brain tumor patients and are willing and eager to have the NIH provide clinical expertise in

making treatment decisions and providing appropriate interpretation of neuro-imaging. Patients will also have timely access to NOB clinical trials for which they may be eligible at the time of disease progression.

The investigational nature and objectives of this trial, the procedures and treatments involved and their attendant risks and discomforts, potential benefits and potential alternative therapies will be carefully explained to the patient, and a signed informed consent document will be obtained by the PI, AI, or clinical staff fellow.

12.6 RISK/BENEFIT ASSESSMENT

12.6.1 Known Potential Risks

12.6.1.1 Non-Physical Risks of Genetic Research

12.6.1.1.1 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. Participants will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with participants, family members or health care providers.

12.6.1.1.2 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 participants, family members or health care providers, this risk will be included in the informed consent document.

12.6.1.1.3 Risk to family or relatives

Family members or relatives may or may not want to be aware of familial tendencies or genetic risks of disease which may cause anxiety about possible future health problems. Participants will be notified of any medically significant and actionable incidental findings. Study results will not be shared with participants.

12.6.1.2 Imaging

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

12.6.1.3 Radiation

Up to 8 whole body CT scans may be performed in a given year on this study, if systemic disease is present. Participants may be exposed to approximately 10.4 rem. This amount is more than would be expected from everyday background radiation. Being exposed to excess radiation can increase the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 1 out of 100 (1.0%) and of getting a fatal cancer is 0.5 out of 100 (0.5%).

12.6.1.4 Blood Collection

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

12.6.1.5 Questionnaires risks

Questionnaires may contain questions that are sensitive in nature.

12.6.1.6 EKG

Other than possibly experiencing some minor skin irritation from the electrodes, there are no anticipated risks related to complete the electrocardiogram and/or the echocardiogram

12.6.2 Known Potential Benefits

The potential benefit to a participant on this study is a reduction in the bulk of their tumor and improvement in cancer lesions, or prolongation of the time that the tumor remains stable without further growth, which may or may not have favorable impact on symptoms and/or survival.

12.7 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 site approved remote platforms used in compliance with local 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 participant 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 on the electronic document. Signatures on electronic documents. When required, witness signatures will be obtained similarly as described for the investigator and participant.

For Lead NCI site at NIH Clinical Center:

Manual (non-electronic) signature on electronic document:

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 subject 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.

For NCI-CONNECT participating sites (non-NIH sites):

Please follow local institutional policies for electronic signature requirements.

12.7.1 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 **12.7**.

12.7.2 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.3.1** 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, 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 the 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

Each 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 Council for 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 (NCI SITE ONLY)

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 NIH 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/each 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-CONNECT Coordinating Center. 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 the clinical sites and by NCI-CONNECT Coordinating Center research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NCI-CONNECT Coordinating Center.

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 Amino Acid Sequence

4 polypeptide chains, which include 2 identical heavy chains with 440 amino acids and 2 identical light chains.

14.1.2 Other Names

BMS-936558, MDX1106, ONO-4538, anti PD-1, BMS-936558-01 Solution for Injection

14.1.3 Classification

Anti-PD-1MAb

14.1.4 M.W.

146,221 daltons

14.1.5 Mode of Action

Nivolumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the downregulation of lymphocyte activation. Nivolumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes

immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.

14.1.6 Description

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.

14.1.7 How Supplied

Nivolumab is supplied by Bristol-Myers Squibb as 100 mg vials (10 mg/mL) with a 0.7 mL overfill. It is supplied in 10 mL type I flint glass vials, with butyl rubber stoppers and aluminum seals.

14.1.8 Preparation

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 0.35 mg/mL. When the dose is fixed (e.g., 240 mg or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL.

14.1.9 Storage

Vials of Nivolumab injection must be stored at 2°-8°C (36°-46°F) and protected from light, freezing, and shaking. The unopened vials can be stored at room temperature (up to 25C, 77F) and room light for up to 48 hours.

Note: **Destruction of nivolumab provided by BMS**: On-site destruction of unused vials of investigational nivolumab should be conducted according to institutional standards. A copy of the drug destruction certificate must be maintained for provision to BMS at the end of the study and a copy must also be sent to the NCI-CONNECT Coordinating Center for sponsor review.

14.1.10 Stability

Shelf-life surveillance of the intact vials is ongoing.

14.1.11 Administration

Following the preparation of nivolumab injection, it is preferred to administer the drug product immediately. If not used immediately, the infusion solution may be stored under refrigeration conditions (2C to 8C, 36F to 46F) and protected from light for up to 7 days, including the product administration period or as described in the instructions provided to the clinical site. The infusion solution may be stored at room temperature (up to 25C, 77F) and room light for a maximum of 8 hours, including the product administration period.

CAUTION: The single-use dosage form contains no antibacterial preservative or bacteriostatic agent. Therefore, it is advised that the product be discarded 8 hours after initial entry.

14.1.12 Route of Administration

Intravenous infusion over 30-60 minutes. The IB shows no significant differences in safety/efficacy between 30 and 60 min infusions; allowing for an administration time slightly

longer than 30 minutes, based on patient tolerability. Do not administer as an IV push or bolus injection.

14.1.13 Method of Administration

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

14.1.14 Toxicities

Summary of the safety profile (81) for 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. See Section **1.2.4** and Nivolumab Investigator's Brochure.

In the pooled dataset of nivolumab 3 mg/kg as monotherapy across tumour types (CA209066, CA209037, CA209067 (monotherapy group only), CA209017, CA209057, CA209063 and CA209025), the most frequent adverse reactions (_ 10%) were fatigue (34%), rash (19%), pruritus (14%), diarrhea (13%), nausea (13%), and decreased appetite (10%). The majority of adverse reactions were mild to moderate (Grade 1 or 2). The following related adverse events have been reported with nivolumab monotherapy at an incidence of > 5%: fatigue, rash, pruritis, diarrhea, nausea, hypothyroidism, decreased appetite, and musculoskeletal pain. Immune-related adverse events have also been reported and may be severe or life-threating.

Tabulated summary of adverse reactions:

Adverse reactions reported in the pooled dataset for patients treated with nivolumab monotherapy. in over 10.000 participants are presented in **Table 3** "Adverse reactions in clinical trials with nivolumab monotherapy". These reactions are presented by system organ class and by frequency. Frequencies are defined as: very common (_ 1/10); common (_ 1/100 to < 1/10); uncommon (_ 1/1,000 to < 1/100); rare (_ 1/10,000 to < 1/1,000); very rare (< 1/10,000). Within each frequency grouping, adverse reactions are presented in the order of decreasing seriousness.

Table 3: Adverse reactions in clinical trials with nivolumab monotherapy	
Infections and infestations	
Common	upper respiratory tract infection
Uncommon	pneumonia[a], bronchitis
Neoplasms benign, malignant and unspecified (including cysts and polyps)	
Rare	histocytic necrotising lymphadenitis (Kikuchi lymphadenitis)
Blood and lymphatic system disorders	
Uncommon	Eosinophilia, thrombocytopenic purpura
Common	Thrombocytopenia, anemia, neutropenia
Immune system disorders	
Common	infusion related reaction[b], hypersensitivity
Uncommon	anaphylactic reaction[b]
Endocrine disorders	
Common	hypothyroidism, hyperthyroidism, hyperglycaemia[b]

Uncommon	adrenal insufficiency, hypopituitarism, hypophysitis, thyroiditis, diabetic ketoacidosis
Rare	diabetes mellitus
Metabolism and nutrition disorders	
Very	decreased appetite
Uncommon	dehydration, metabolic acidosis
Hepatobiliary disorders	
Uncommon	hepatitis[b], hyperbilirubinemia
Rare	Cholestasis
Nervous system disorders	
Common	peripheral neuropathy, headache, dizziness
Uncommon	Polyneuropathy
Rare	Guillain-Barré syndrome, demyelination, myasthenic syndrome, autoimmune neuropathy (including facial, acoustic and abducens nerve paresis)
Eye disorders	
Common	vision blurred, dry eye
Uncommon	Uveitis
Cardiac disorders	
Uncommon	Tachycardia
Rare	arrhythmia (including ventricular arrhythmia)[c], atrial fibrillation
Vascular disorders	
Common	Hypertension
Uncommon	Vasculitis
Respiratory, thoracic, and mediastinal disorders	
Common	pneumonitis[a,b], dyspnoea, cough
Uncommon	pleural effusion
Rare	lung infiltration
Gastrointestinal disorders	
Very	diarrhoea, nausea
Common	colitis, stomatitis, vomiting, abdominal pain, constipation, dry mouth
Uncommon	Pancreatitis
Rare	gastritis, duodenal ulcer
Skin and subcutaneous tissue disorders	
Very common	rash[d], pruritus
Common	vitiligo, dry skin, erythema, alopecia
Uncommon	erythema multiforme, psoriasis, rosacea, urticaria
Rare	toxic epidermal necrolysis[a,e]
Musculoskeletal and connective tissue disorders	
Common	musculoskeletal pain[f], arthralgia
Uncommon	polymyalgia rheumatica, arthritis
Rare	Myopathy
Renal and urinary disorders	
Uncommon	tubulointerstitial nephritis, renal failure[a,b]
General disorders and administration site conditions	
Very	Fatigue

Common	Insomnia, pyrexia, oedema (including peripheral oedema)
Uncommon	pain, chest pain
Investigations[g]	
Rare	Increased blood creatine phosphokinase (CPK), and increased Gamma-glutamyl transferase
Very common	increased AST, increased ALT, increased alkaline phosphatase, increased lipase, increased amylase, hypocalcaemia, increased creatinine, lymphopaenia, leucopoenia, thrombocytopaenia, anaemia, hypercalcaemia, hyperkalaemia, hypokalaemia, hypomagnesaemia, hyponatraemia
Common	increased total bilirubin, neutropaenia, hypermagnesaemia, hypernatraemia,

[a] Fatal cases have been reported in completed or ongoing clinical studies
[b] Life-threatening cases have been reported in completed or ongoing clinical studies.
[c] The frequency of adverse events in the cardiac disorders system organ class regardless of causality was higher in the nivolumab group than in the chemotherapy group in post-CTLA4/BRAF inhibitor metastatic melanoma population. Incidence rates per 100 person-years of exposure were 9.3 vs 0; serious cardiac events were reported by 4.9% patients in the nivolumab group vs 0 in the investigator´s choice group. The frequency of cardiac adverse events was lower in the nivolumab group than in the dacarbazine group in the metastatic melanoma without prior treatment population. All were considered not related to nivolumab by investigators except arrhythmia (atrial fibrillation, tachycardia and ventricular arrhythmia).
[d] Rash is a composite term which includes maculopapular rash, rash erythematous, rash pruritic, rash follicular, rash macular, rash morbilliform, rash papular, rash pustular, rash papulosquamous, rash vesicular, rash generalised, dermatitis, dermatitis acneiform, dermatitis allergic, dermatitis atopic, dermatitis bullous, dermatitis exfoliative, dermatitis psoriasiform, and drug eruption.
[e] Reported in studies outside the pooled dataset. The frequency is based on the program-wide exposure.
[f] Musculoskeletal pain is a composite term which includes back pain, bone pain, musculoskeletal chest pain, musculoskeletal discomfort, myalgia, neck pain, pain in extremity, spinal pain.
[g] Frequencies reflect the proportion of patients who experienced a worsening from baseline in laboratory measurements.

14.1.15 Potential Drug Interactions

No incompatibilities between Nivolumab injection and polyvinyl chloride (PVC), non-PVC/non-DEHP (di[2-ethylhexyl]phthalate) IV components, or glass bottles have been observed.

15 REFERENCES

References

1. Louis DN, Ohgaki H, Wiestler OD, Cavanee WK, Ellison DW, Figarella-Branger D, et al. WHO Classification of Tumours of the Central Nervous System. Bosman F, Jaffee ES, Lakhani SR, Ohgaki H, editors. Lyon: International Agency for Research on Cancer; 2016.
2. Ostrom QT, Gittleman H, Liao P, Rouse C, Chen Y, Dowling J, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011. Neuro Oncol. 2014;16 Suppl 4:iv1-63.
3. Pajtler KW, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F, et al. Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups. Cancer Cell. 2015;27(5):728-43.
4. Wani K, Armstrong TS, Vera-Bolanos E, Raghunathan A, Ellison D, Gilbertson R, et al. A prognostic gene expression signature in infratentorial ependymoma. Acta Neuropathol. 2012;123(5):727-38.
5. Mack SC, Witt H, Piro RM, Gu L, Zuyderduyn S, Stutz AM, et al. Epigenomic alterations define lethal CIMP-positive ependymomas of infancy. Nature. 2014;506(7489):445-50.
6. Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y, et al. C11orf95-RELA fusions drive oncogenic NF-kappaB signalling in ependymoma. Nature. 2014;506(7489):451-5.
7. Vera-Bolanos E, Aldape K, Yuan Y, Wu J, Wani K, Necesito-Reyes MJ, et al. Clinical course and progression-free survival of adult intracranial and spinal ependymoma patients. Neuro Oncol. 2015;17(3):440-7.
8. Mack SC, Agnihotri S, Bertrand KC, Wang X, Shih DJ, Witt H, et al. Spinal Myxopapillary Ependymomas Demonstrate a Warburg Phenotype. Clin Cancer Res. 2015;21(16):3750-8.
9. Ghasemi DR, Sill M, Okonechnikov K, Korshunov A, Yip S, Schutz PW, et al. MYCN amplification drives an aggressive form of spinal ependymoma. Acta Neuropathol. 2019;138(6):1075-89.
10. Swanson AA, Raghunathan A, Jenkins RB, Messing-Jünger M, Pietsch T, Clarke MJ, et al. Spinal Cord Ependymomas With MYCN Amplification Show Aggressive Clinical Behavior. Journal of neuropathology and experimental neurology. 2019;78(9):791-7.
11. Raffeld M, Abdullaev Z, Pack SD, Xi L, Nagaraj S, Briceno N, et al. High level MYCN amplification and distinct methylation signature define an aggressive subtype of spinal cord ependymoma. Acta Neuropathologica Communications. 2020;8(1):101.
12. Armstrong TS, Vera-Bolanos E, Bekele BN, Aldape K, Gilbert MR. Adult ependymal tumors: prognosis and the M. D. Anderson Cancer Center experience. Neuro Oncol. 2010;12(8):862-70.
13. Gilbert MR, Ruda R, Soffietti R. Ependymomas in adults. Curr Neurol Neurosci Rep. 2010;10(3):240-7.
14. Brandes AA, Cavallo G, Reni M, Tosoni A, Nicolardi L, Scopece L, et al. A multicenter retrospective study of chemotherapy for recurrent intracranial ependymal tumors in adults by the Gruppo Italiano Cooperativo di Neuro-Oncologia. Cancer. 2005;104(1):143-8.

15. Gilbert MR, Yuan Y, Wani K, Wu J, Omuro A, Lieberman F, et al. A Phase II study of lapatinib and dose-dense temozolomide for adults with recurrent ependymoma: A CERN cllinical trial. Neuro Oncol. 2014;16(v13).
16. Zhao F, Ohgaki H, Xu L, Giangaspero F, Li C, Li P, et al. Molecular subgroups of adult medulloblastoma: a long-term single-institution study. Neuro Oncol. 2016;18(7):982-90.
17. Robinson GW, Orr BA, Wu G, Gururangan S, Lin T, Qaddoumi I, et al. Vismodegib Exerts Targeted Efficacy Against Recurrent Sonic Hedgehog-Subgroup Medulloblastoma: Results From Phase II Pediatric Brain Tumor Consortium Studies PBTC-025B and PBTC-032. J Clin Oncol. 2015;33(24):2646-54.
18. CBTRUS. 2005-2006 Statistical Report: Primary Brain Tumors in the United States Statistical Report, 1998-2002 (Years Data Collected) 2006 [Available from: http://www.cbtrus.org/2005-2006/tables/2006.table8.pdf.
19. Miller S, Rogers HA, Lyon P, Rand V, Adamowicz-Brice M, Clifford SC, et al. Genome-wide molecular characterization of central nervous system primitive neuroectodermal tumor and pineoblastoma. Neuro Oncol. 2011;13(8):866-79.
20. Tate M, Sughrue ME, Rutkowski MJ, Kane AJ, Aranda D, McClinton L, et al. The long-term postsurgical prognosis of patients with pineoblastoma. Cancer. 2012;118(1):173-9.
21. Selvanathan SK, Hammouche S, Smethurst W, Salminen HJ, Jenkinson MD. Outcome and prognostic features in adult pineoblastomas: analysis of cases from the SEER database. Acta Neurochir (Wien). 2012;154(5):863-9.
22. Fauchon F, Jouvet A, Paquis P, Saint-Pierre G, Mottolese C, Ben Hassel M, et al. Parenchymal pineal tumors: a clinicopathological study of 76 cases. Int J Radiat Oncol Biol Phys. 2000;46(4):959-68.
23. Jouvet A, Saint-Pierre G, Fauchon F, Privat K, Bouffet E, Ruchoux MM, et al. Pineal parenchymal tumors: a correlation of histological features with prognosis in 66 cases. Brain Pathol. 2000;10(1):49-60.
24. Lancia A, Becherini C, Detti B, Bottero M, Baki M, Cancelli A, et al. Radiotherapy for papillary tumor of the pineal region: A systematic review of the literature. Clinical neurology and neurosurgery. 2020;190:105646.
25. Merino DM, Shlien A, Villani A, Pienkowska M, Mack S, Ramaswamy V, et al. Molecular characterization of choroid plexus tumors reveals novel clinically relevant subgroups. Clin Cancer Res. 2015;21(1):184-92.
26. Thomas C, Sill M, Ruland V, Witten A, Hartung S, Kordes U, et al. Methylation profiling of choroid plexus tumors reveals 3 clinically distinct subgroups. Neuro Oncol. 2016;18(6):790-6.
27. Walcott BP, Nahed BV, Mohyeldin A, Coumans JV, Kahle KT, Ferreira MJ. Chordoma: current concepts, management, and future directions. Lancet Oncol. 2012;13(2):e69-76.
28. McMaster ML, Goldstein AM, Bromley CM, Ishibe N, Parry DM. Chordoma: incidence and survival patterns in the United States, 1973-1995. Cancer Causes Control. 2001;12(1):1-11.
29. Williams BJ, Raper DM, Godbout E, Bourne TD, Prevedello DM, Kassam AB, et al. Diagnosis and treatment of chordoma. J Natl Compr Canc Netw. 2013;11(6):726-31.
30. Mathios D, Ruzevick J, Jackson CM, Xu H, Shah S, Taube JM, et al. PD-1, PD-L1, PD-L2 expression in the chordoma microenvironment. J Neurooncol. 2015;121(2):251-9.
31. Feng Y, Shen J, Gao Y, Liao Y, Cote G, Choy E, et al. Expression of programmed cell death ligand 1 (PD-L1) and prevalence of tumor-infiltrating lymphocytes (TILs) in chordoma. Oncotarget. 2015;6(13):11139-49.

32. Chen S, Tanaka S, Giannini C, Morris J, Yan ES, Buckner J, et al. Gliomatosis cerebri: clinical characteristics, management, and outcomes. J Neurooncol. 2013;112(2):267-75.
33. Guillamo JS, Monjour A, Taillandier L, Devaux B, Varlet P, Haie-Meder C, et al. Brainstem gliomas in adults: prognostic factors and classification. Brain. 2001;124(Pt 12):2528-39.
34. Babu R, Kranz PG, Agarwal V, McLendon RE, Thomas S, Friedman AH, et al. Malignant brainstem gliomas in adults: clinicopathological characteristics and prognostic factors. J Neurooncol. 2014;119(1):177-85.
35. Kesari S, Kim RS, Markos V, Drappatz J, Wen PY, Pruitt AA. Prognostic factors in adult brainstem gliomas: a multicenter, retrospective analysis of 101 cases. J Neurooncol. 2008;88(2):175-83.
36. Grimm SA, Chamberlain MC. Brainstem glioma: a review. Curr Neurol Neurosci Rep. 2013;13(5):346.
37. Theeler BJ, Ellezam B, Melguizo-Gavilanes I, de Groot JF, Mahajan A, Aldape KD, et al. Adult brainstem gliomas: Correlation of clinical and molecular features. J Neurol Sci. 2015;353(1-2):92-7.
38. Feng J, Hao S, Pan C, Wang Y, Wu Z, Zhang J, et al. The H3.3 K27M mutation results in a poorer prognosis in brainstem gliomas than thalamic gliomas in adults. Hum Pathol. 2015;46(11):1626-32.
39. Zhang RQ, Shi Z, Chen H, Chung NY, Yin Z, Li KK, et al. Biomarker-based prognostic stratification of young adult glioblastoma. Oncotarget. 2016;7(4):5030-41.
40. Gessi M, Giangaspero F, Pietsch T. Atypical teratoid/rhabdoid tumors and choroid plexus tumors: when genetics "surprise" pathology. Brain Pathol. 2003;13(3):409-14.
41. Shonka NA, Armstrong TS, Prabhu SS, Childress A, Choi S, Langford LA, et al. Atypical teratoid/rhabdoid tumors in adults: a case report and treatment-focused review. J Clin Med Res. 2011;3(2):85-92.
42. Tekautz TM, Fuller CE, Blaney S, Fouladi M, Broniscer A, Merchant TE, et al. Atypical Teratoid/Rhabdoid Tumors (ATRT): Improved Survival in Children 3 Years of Age and Older With Radiation Therapy and High-Dose Alkylator-Based Chemotherapy. J Clin Oncol. 2005;23(7):1491-9.
43. Hwang K, Koh EJ, Choi EJ, Kang TH, Han JH, Choe G, et al. PD-1/PD-L1 and immune-related gene expression pattern in pediatric malignant brain tumors: clinical correlation with survival data in Korean population. J Neurooncol. 2018;139(2):281-91.
44. Rogers L, Barani I, Chamberlain M, Kaley TJ, McDermott M, Raizer J, et al. Meningiomas: knowledge base, treatment outcomes, and uncertainties. A RANO review. J Neurosurg. 2015;122(1):4-23.
45. Brastianos PK, Horowitz PM, Santagata S, Jones RT, McKenna A, Getz G, et al. Genomic sequencing of meningiomas identifies oncogenic SMO and AKT1 mutations. Nat Genet. 2013;45(3):285-9.
46. Du Z, Abedalthagafi M, Aizer AA, McHenry AR, Sun HH, Bray MA, et al. Increased expression of the immune modulatory molecule PD-L1 (CD274) in anaplastic meningioma. Oncotarget. 2015;6(7):4704-16.
47. Cachia D, Kamiya-Matsuoka C, Mandel JJ, Olar A, Cykowski MD, Armstrong TS, et al. Primary and secondary gliosarcomas: clinical, molecular and survival characteristics. J Neurooncol. 2015;125(2):401-10.

48. Walker GV, Gilbert MR, Prabhu SS, Brown PD, McAleer MF. Temozolomide use in adult patients with gliosarcoma: an evolving clinical practice. J Neurooncol. 2013;112(1):83-9.
49. McAleer MF, Brown PD. Therapeutic management of gliosarcoma in the temozolomide era. CNS Oncol. 2015;4(3):171-8.
50. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131(6):803-20.
51. Ida CM, Rodriguez FJ, Burger PC, Caron AA, Jenkins SM, Spears GM, et al. Pleomorphic Xanthoastrocytoma: Natural History and Long-Term Follow-Up. Brain Pathol. 2015;25(5):575-86.
52. Sturm D, Orr BA, Toprak UH, Hovestadt V, Jones DTW, Capper D, et al. New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs. Cell. 2016;164(5):1060-72.
53. Agata Y, Kawasaki A, Nishimura H, Ishida Y, Tsubata T, Yagita H, et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int Immunol. 1996;8(5):765-72.
54. Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer--response. Clin Cancer Res. 2013;19(19):5542.
55. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med. 1999;5(12):1365-9.
56. Mazanet MM, Hughes CC. B7-H1 is expressed by human endothelial cells and suppresses T cell cytokine synthesis. J Immunol. 2002;169(7):3581-8.
57. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A. 2002;99(19):12293-7.
58. Strome SE, Dong H, Tamura H, Voss SG, Flies DB, Tamada K, et al. B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. Cancer Res. 2003;63(19):6501-5.
59. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res. 2005;65(3):1089-96.
60. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol. 2010;28(19):3167-75.
61. Weber JS, Kudchadkar RR, Yu B, Gallenstein D, Horak CE, Inzunza HD, et al. Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naive melanoma. J Clin Oncol. 2013;31(34):4311-8.
62. Zeng J, See AP, Phallen J, Jackson CM, Belcaid Z, Ruzevick J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys. 2013;86(2):343-9.
63. Topalian SL, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol. 2014;32(10):1020-30.
64. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for

Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000;92(3):205-16.
65. Reardon DA, Sampson JH, Sahebjam S, Lim M, Baehring JM, Vlahovic G, et al. Safety and activity of nivolumab (nivo) monotherapy and nivo in combination with ipilimumab (ipi) in recurrent glioblastoma (GBM): Updated results from checkmate-143. 2016;34(15_suppl):2014-.
66. Armstrong TS, Gning I, Mendoza TR, Vera-Bolanos E, Gilbert MR, Rhines LD, et al. Reliability and validity of the M. D. Anderson Symptom Inventory-Spine Tumor Module. J Neurosurg Spine. 2010;12(4):421-30.
67. Armstrong TS, Mendoza T, Gning I, Coco C, Cohen MZ, Eriksen L, et al. Validation of the M.D. Anderson Symptom Inventory Brain Tumor Module (MDASI-BT). J Neurooncol. 2006;80(1):27-35.
68. Desai A, Gainor JF, Hegde A, Schram AM, Curigliano G, Pal S, et al. COVID-19 vaccine guidance for patients with cancer participating in oncology clinical trials. Nature reviews Clinical oncology. 2021;18(5):313-9.
69. Daud AI, Loo K, Pauli ML, Sanchez-Rodriguez R, Sandoval PM, Taravati K, et al. Tumor immune profiling predicts response to anti–PD-1 therapy in human melanoma. The Journal of Clinical Investigation. 2016;126(9):3447-52.
70. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. 2015;372(26):2509-20.
71. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 2015;160(1-2):48-61.
72. Ku GY, Yuan J, Page DB, Schroeder SE, Panageas KS, Carvajal RD, et al. Single-institution experience with ipilimumab in advanced melanoma patients in the compassionate use setting: lymphocyte count after 2 doses correlates with survival. Cancer. 2010;116(7):1767-75.
73. Tassignon J, Burny W, Dahmani S, Zhou L, Stordeur P, Byl B, et al. Monitoring of cellular responses after vaccination against tetanus toxoid: comparison of the measurement of IFN-gamma production by ELISA, ELISPOT, flow cytometry and real-time PCR. Journal of immunological methods. 2005;305(2):188-98.
74. Callan MFC, Annels N, Steven N, Tan L, Wilson J, McMichael AJ, et al. T cell selection during the evolution of CD8+ T cell memory in vivo. 1998;28(12):4382-90.
75. Lalvani A, Brookes R, Hambleton S, Britton WJ, Hill AV, McMichael AJ. Rapid effector function in CD8+ memory T cells. The Journal of experimental medicine. 1997;186(6):859-65.
76. Collaboration A, Aad G, Abajyan T, Abbott B, Abdallah J, Abdel Khalek S, et al. Search for Magnetic Monopoles in $\sqrt{s}\mathbf{=}7\text{ }\text{ }\mathrm{TeV}$ pp Collisions with the ATLAS Detector. Physical Review Letters. 2012;109(26):261803.
77. Sim GC, Martin-Orozco N, Jin L, Yang Y, Wu S, Washington E, et al. IL-2 therapy promotes suppressive ICOS+ Treg expansion in melanoma patients. J Clin Invest. 2014;124(1):99-110.
78. Gros A, Parkhurst MR, Tran E, Pasetto A, Robbins PF, Ilyas S, et al. Prospective identification of neoantigen-specific lymphocytes in the peripheral blood of melanoma patients. Nat Med. 2016;22(4):433-8.
79. Schultze JL, Michalak S, Seamon MJ, Dranoff G, Jung K, Daley J, et al. CD40-activated human B cells: an alternative source of highly efficient antigen presenting cells to generate autologous antigen-specific T cells for adoptive immunotherapy. The Journal of Clinical Investigation. 1997;100(11):2757-65.

80. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol. 2017;18(3):e143-e52.
81. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol. 2010;28(11):1963-72.
82. Okada H, Weller M, Huang R, Finocchiaro G, Gilbert MR, Wick W, et al. Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. Lancet Oncol. 2015;16(15):e534-e42.
83. Zhou H, Lee JJ, Yuan Y. BOP2: Bayesian optimal design for phase II clinical trials with simple and complex endpoints. Stat Med. 2017;36(21):3302-14.
84. Cai C, Liu S, Yuan Y. A Bayesian design for phase II clinical trials with delayed responses based on multiple imputation. Stat Med. 2014;33(23):4017-28.

16 APPENDICES

16.1 QUESTIONNAIRES (MDASI-BT & MDASI-SP)

Participants will complete and return Patient-Reported Outcomes (PRO) questionnaires. These questionnaires may be found in the separate Study Instrument Packet.

16.2 PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	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.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

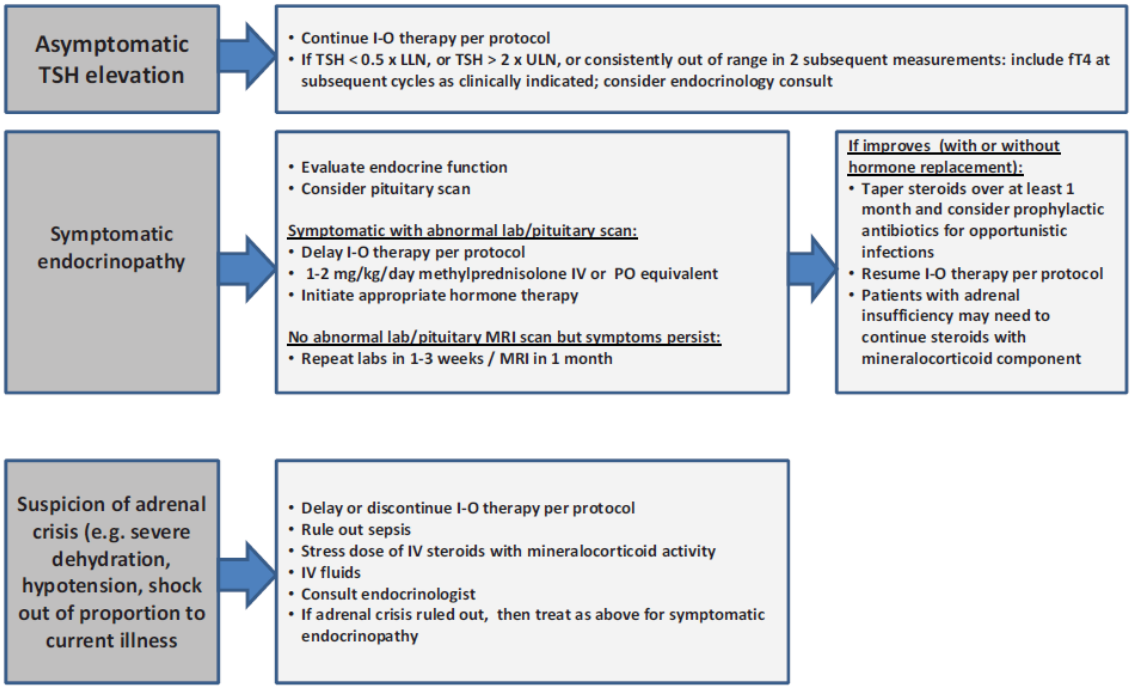
16.3 NEUROLOGIC EVALUATION

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

16.4 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

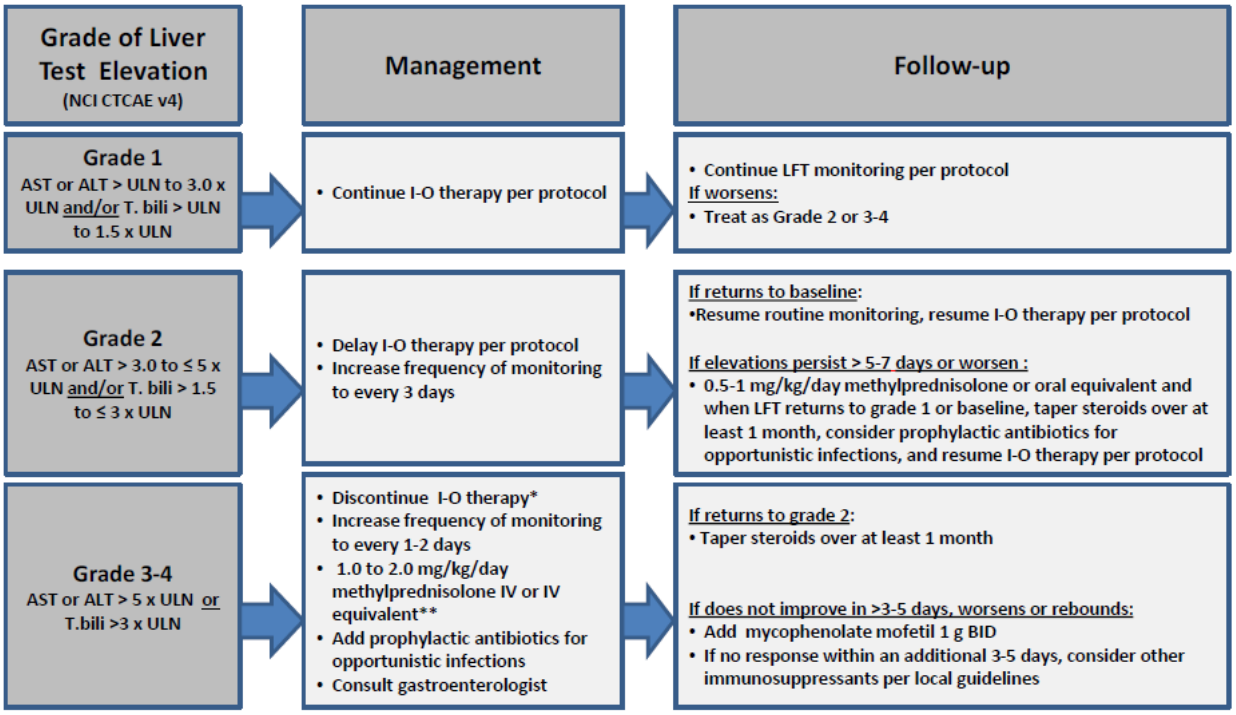
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

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

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

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.



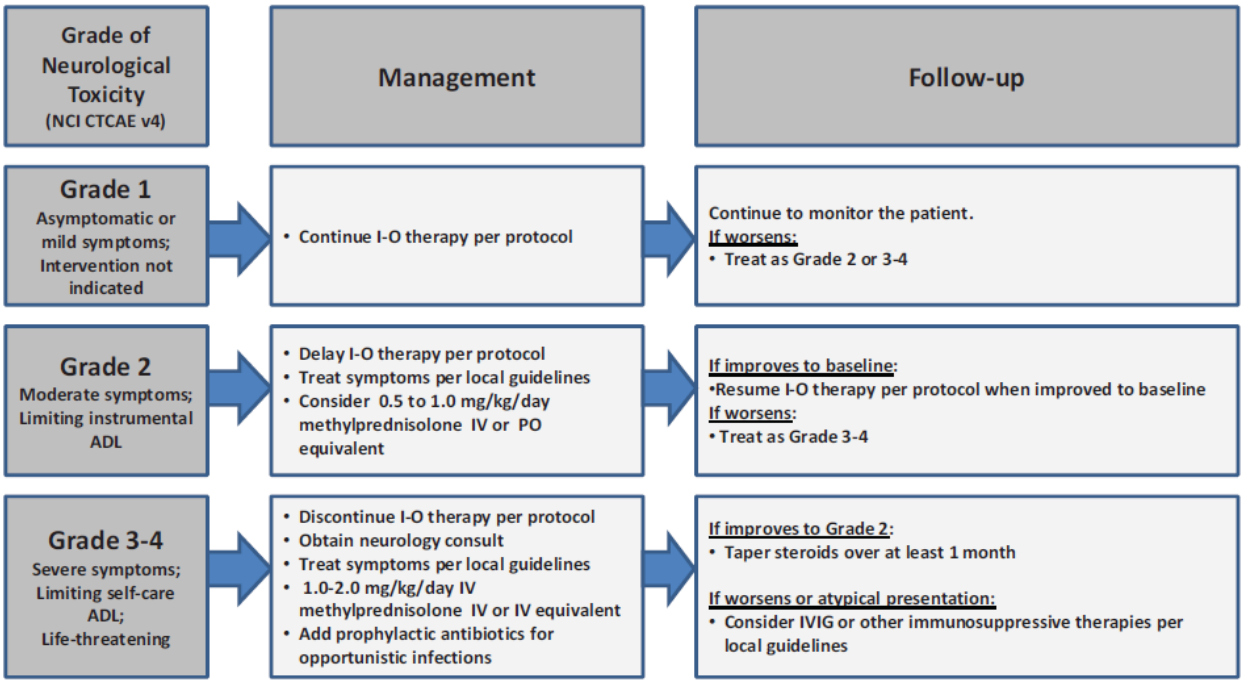
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

Neurological Adverse Event Management Algorithm

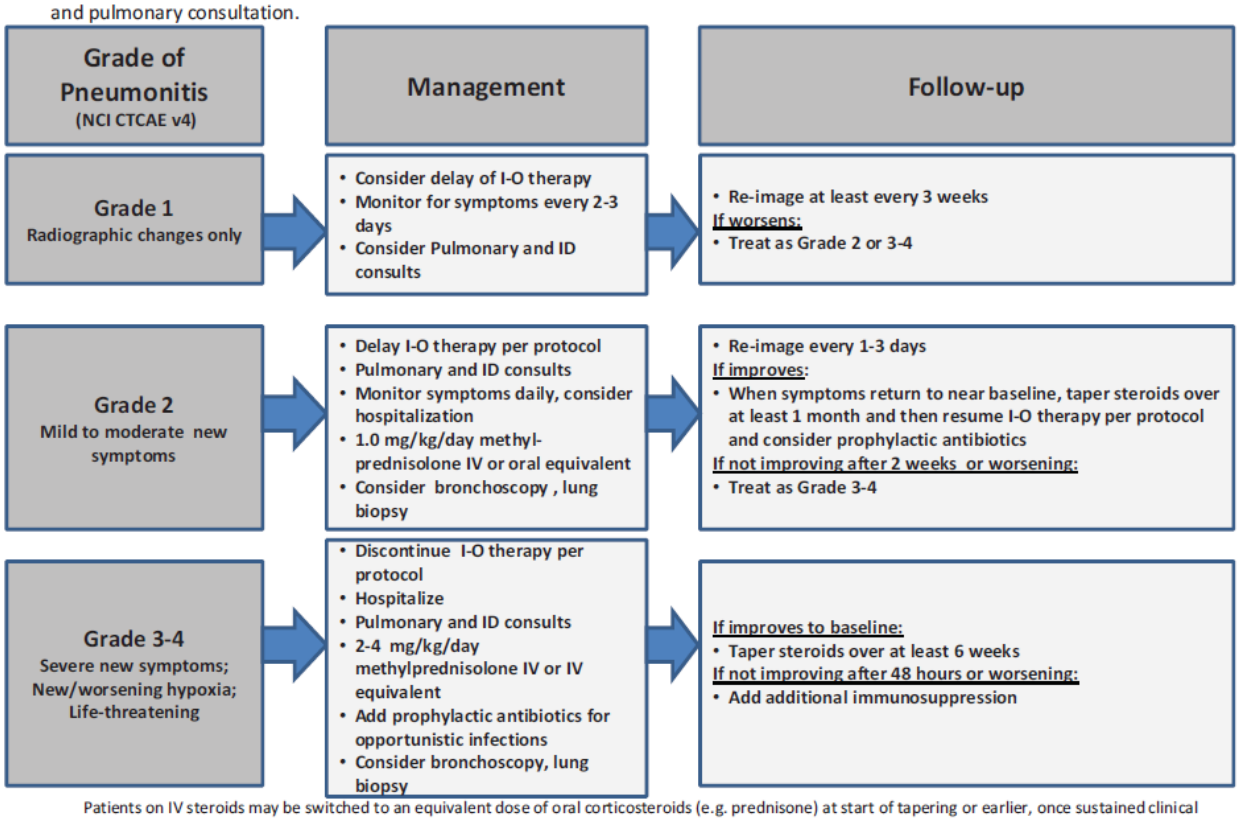
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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.

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

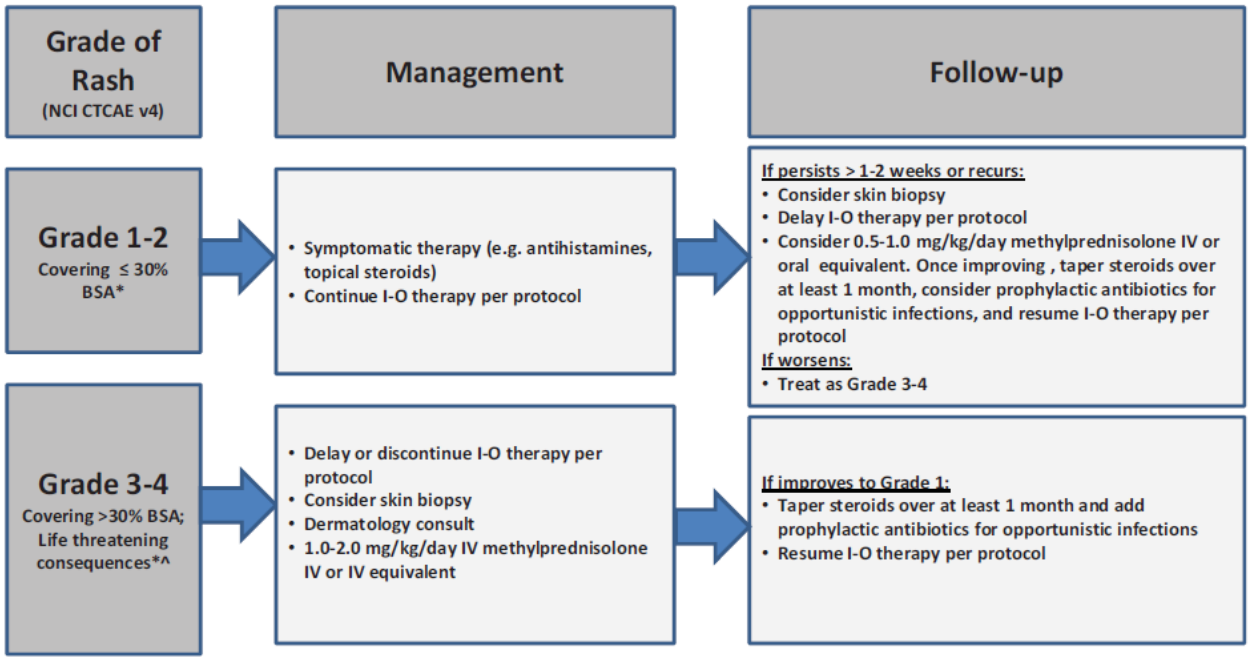
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy

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.



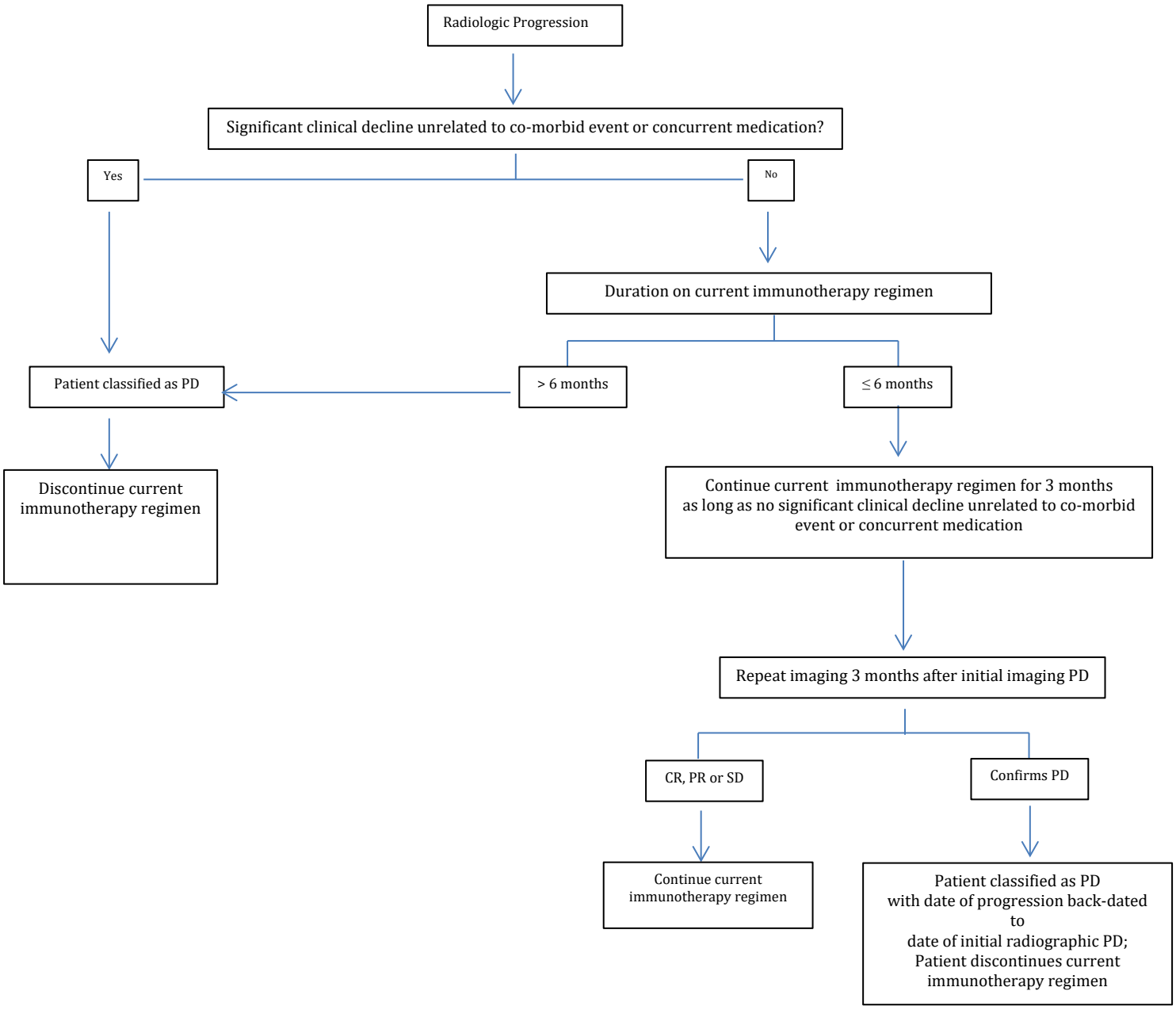
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

iRANO Criteria

Table 1

RANO Criteria

	Malignant Glioma[16]	Low-Grade Glioma[17]	Brain Metastases[18]
Complete Response	-Disappearance of all enhancing disease for ≥ 4 weeks *AND* - No new lesions *AND* - Stable/improved T2/FLAIR *AND* - No more than physiologic steroids *AND* - Stable/improved clinically	-Disappearance of all enhancing and T2/FLAIR disease for ≥ 4 weeks *AND* - No new lesions *AND* - No more than physiologic steroids *AND* - Stable/improved clinically	-Disappearance of all enhancing target and non-target lesions for ≥ 4 weeks *AND* - No new lesions *AND* - No steroids *AND* - Stable/improved clinically
Partial Response	- ≥ 50% ↓ sum of biperpendicular diameters of enhancing disease for ≥ 4 weeks *AND* - No new lesions *AND* - Stable/improved T2/FLAIR *AND* - Stable/improved steroids *AND* - Stable/improved clinically	- ≥ 50% ↓ sum of biperpendicular diameters of T2/FLAIR disease for ≥ 4 weeks *AND* - No new lesions *AND* - Stable/improved steroids *AND* - Stable/improved clinically	- ≥ 30% ↓ sum of longest diameters of target lesions for ≥ 4 weeks *AND* - No new lesions *AND* - Stable/improved steroids *AND* - Stable/improved clinically
Minor Response	- Non-applicable	- 25-49% ↓ sum of biperpendicular diameters of T2/FLAIR disease for ≥ 4 weeks *AND* - No new lesions *AND* - Stable/improved clinically	Not applicable
Stable Disease	- Does not qualify for CR, PR, PD *AND* - No new lesions *AND* - Stable/improved T2/FLAIR *AND* - Stable/improved steroids *AND* - Stable/improved clinically	- Does not qualify for CR, PR, PD *AND* - No new lesions *AND* - Stable/improved T2/FLAIR *AND* - Stable/improved steroids *AND* - Stable/improved clinically	- Does not qualify for CR, PR, PD
Progressive Disease	- ≥ 25% ↑ sum of biperpendicular diameters of enhancing disease *OR* - New lesions *OR* - Significant worsened T2/FLAIR *OR* - Significant clinical decline	- ≥ 25% ↑ sum of biperpendicular diameters of T2/FLAIR disease *OR* - New lesions *OR* - Significant clinical decline	- ≥ 20% ↑ sum of longest diameters of target lesions *OR* -Unequivocal progression of enhancing non-target lesions *OR* - New lesions *OR* - Significant clinical decline
iRANO	Confirmation of progression on follow-up imaging 3 months after initial radiographic progression if: 1. No new or significantly worsened neurologic deficits not due to co-morbid event or concurrent medication *AND* 2. ≤ 6 months from initiation of immunotherapy If follow-up imaging confirms progression, the date of actual progression should be back-dated to the date of initial radiographic progression		


Radiologic Progression
Significant clinical decline unrelated to co-morbid event or concurrent medication?
Yes
No
Duration on current immunotherapy regimen
Patient classified as PD
> 6 months
≤ 6 months
Discontinue current immunotherapy regimen
Continue current immunotherapy regimen for 3 months as long as no significant clinical decline unrelated to co-morbid event or concurrent medication
Repeat imaging 3 months after initial imaging PD
CR, PR or SD
Confirms PD
Continue current immunotherapy regimen
Patient classified as PD with date of progression back-dated to date of initial radiographic PD; Patient discontinues current immunotherapy regimen

16.5 iRECIST

Comparison of RECIST 1.1 and iRECIST

	RECIST 1.1	iRECIST
Definitions of measurable and non-measurable disease; numbers and site of target disease	Measurable lesions are ≥10 mm in diameter (≥15 mm for nodal lesions); maximum of five lesions (two per organ); all other disease is considered non-target (must be ≥10 mm in short axis for nodal disease)	No change from RECIST 1.1; however, new lesions are assessed as per RECIST 1.1 but are recorded separately on the case report form (but not included in the sum of lesions for target lesions identified at baseline)
Complete response, partial response, or stable disease	Cannot have met criteria for progression before complete response, partial response, or stable disease	Can have had iUPD (one or more instances), but not iCPD, before iCR, iPR, or iSD
Confirmation of complete response or partial response	Only required for non-randomised trials	As per RECIST 1.1
Confirmation of stable disease	Not required	As per RECIST 1.1
New lesions	Result in progression; recorded but not measured	Results in iUPD but iCPD is only assigned on the basis of this category if at next assessment additional new lesions appear or an increase in size of new lesions is seen (≥5 mm for sum of new lesion target or any increase in new lesion non-target); the appearance of new lesions when none have previously been recorded, can also confirm iCPD
Independent blinded review and central collection of scans	Recommended in some circumstances —eg, in some trials with progression-based endpoints planned for marketing approval	Collection of scans (but not independent review) recommended for all trials
Confirmation of progression	Not required (unless equivocal)	Required
Consideration of clinical status	Not included in assessment	Clinical stability is considered when deciding whether treatment is continued after iUPD

"i" indicates immune responses assigned using iRECIST. RECIST=Response Evaluation Criteria in Solid Tumours. iUPD=unconfirmed progression. iCPD=confirmed progression. iCR=complete response. iPR=partial response. iSD=stable disease.

16.6 FLOW CYTOMETRY TECHNIQUES FOR ANALYSIS OF TUMOR AND PERIPHERAL BLOOD

Please note that tubes and media referenced below may be substituted based on availability with the permission of the PI or laboratory investigator. The location of specimen processing or analysis may be adjusted with the permission of the PI or laboratory investigator.
To maximize the cell characteristics captured by flow cytometry, we have developed 18-color panels for immune analysis of tumors and peripheral blood. Once major immune cell subsets are identified using canonical gating strategies, we will quantify the frequency, intensity, and co-expression of epitopes that are regulated in response to immune activation.

Quality control measures:

Tissue: Cell viability and cell number are assessed before cryopreservation and following thaw. Low viability (<85% for blood and <50% for tumor) will preclude functional assays, including cytokine secretion and interferon secretion. Viable cells are quantified using acridine orange/propidium iodide solution to count all nuclei (nuclear acridine orange uptake) and eliminate dead cells (propidium iodide exclusion).
Reagents/equipment: Routine instrument QC, titration of each fluorescent antibody clone, conjugate, and lot. Comparison of different clones and conjugates. Fluorescence minus one (FMO) controls where appropriate.

Sample treatment description:

All tissue samples are processed as quickly as possible to minimize undesired cellular changes due to sample handling. Samples are cryopreserved in 10% DMSO/90% fetal bovine serum and transferred to liquid nitrogen within 72 hours of freezing.
Brain tumor: Samples are weighed, minced, and mechanically dissociated using a Medimachine. Enzymatic dissociation is not used so that cleavage of surface epitopes is minimized. Immune cells are isolated using a Percoll density gradient.
Blood: Blood is drawn into BD Vacutainer cell preparation tubes (CPT or CPB tubes) with 8mL draw capacity. Tubes are kept at room temperature and in a horizontal rotor (swinging bucket) position until processing, as refrigeration can induce granule release from myeloid cells. Blood is processed within 2 hours of drawing. Tubes are centrifuged at 1,800*g for 30 minutes to separate peripheral blood mononuclear cells (PBMCs) and plasma.
Cryorecovery: Samples are thawed rapidly at 37C, freezing medium is removed, and samples are permitted to rest overnight in lymphocyte growth medium (no added growth factors or cytokines). This permits highly autofluorescent apoptotic cells to die, thereby reducing the sample's fluorescent background. Samples are run in batches so that staining and machine performance is consistent across samples.

Instrument: BD LSRII SORP Configuration

Laser	Power	Parameter name	Discriminating filters (DF)	Dichroic Filter (LP Mirror)	Fluorochromes
355 nm UV	**60mW**	**UV740**	**740/35**	**690LP**	**BUV737**

Laser	Power	Parameter name	Discriminating filters (DF)	Dichroic Filter (LP Mirror)	Fluorochromes
		UV515	515/30	450LP	DAPI, Hoechst Blue or BUV496
		UV379	379/28	NA	BUV395
404 nm Violet	100mW	V780	780/60	750LP	BD Horizon™ Brilliant Violet™ 786
		V710	710/50	*695LP*	BD Horizon™ Brilliant Violet™ 711
		V670	670/30	635LP	BD Horizon™ Brilliant Violet™ 650
		V610	610/20	595LP	BD Horizon™ Brilliant Violet™ 605
		V525	525/50	*495LP*	BD Horizon™ Brilliant Violet™ 510, AmCyan, Pacific Orange, V500, Live/Dead Aqua
		V450	450/50	NA	BD Horizon™ Brilliant Violet™ 421, V450, VPD450, Pacific Blue™, DAPI, e450
488 nm Blue	60mW	B695	695/40	635LP	PerCP-Cy 5.5, PE-Cy 5.5
		B530	530/30	505LP	FITC, Alexa Fluor 488, GFP
		SSC	488/10	NA	SSC
561 nm Yellow-Green	50mW	YG780	780/60	750LP	PE-Cy7
		YG710	710/50	685LP	PE-Cy5.5
		YG610	610/20	600 LP	PE-Texas Red, PE-CF594, PE/Dazzle 594, PE-eFluor 610, ECD, mCherry, tdTomato (Option 2), PI
		YG586	586/15	NA	PE, tdTomato (Option 1), DsRed
640 nm Red	40mW	R780	780/60	750LP	APC-Cy7, APC-H7, APCe780
		R730	730/45	*710LP*	Alexa Fluor® 700
		R670	670/30	NA	APC, Alexa Fluor® 647

Immune subsets. Common names and phenotypes are listed. Previous gating strategy is used by not listed (single cells, live, lymphocyte gates).

Cell Name	Identifying Markers	Variable Markers
T cells	$CD3^{+}CD45^{+}CD14^{-}$, CD4, CD8	

Cell Name	Identifying Markers	Variable Markers
Naïve T cell	$CD45RA^{+}$ $CD45RO^{-}$ $CCR7^{+}$ $CCR5^{-}$	PD-1, CTLA-4, TIM3, LAG3 HLA-DR
Memory Stem T cells	$CD45RA^{+}$ $CD45RO^{-}$ $CCR7^{+}$ $CCR5^{-}$ $CD95^{+}$ CD122 (IL-2Rbeta)$^{+}$	PD-1, CTLA-4, TIM3, LAG3 HLA-DR
Central Memory T cell	$CD45RA^{-}$ $CD45RO^{+}$ $CCR7^{+}$ $CCR5^{-}$	PD-1, CTLA-4, TIM3, LAG3 HLA-DR
Effector Memory T cell	$CD45RA^{-}$ $CD45RO^{+}$ $CCR7^{-}$ $CCR5^{+}$	PD-1, CTLA-4, TIM3, LAG3 HLA-DR
Effector T cell	$CD45RA^{+}$ $CD45RO^{-}$ $CCR7^{-}$ $CCR5^{+}$	PD-1, CTLA-4, TIM3, LAG3 HLA-DR
Th1 T cell	$CXCR3^{+}$ $IFNg^{+}$ $IL\text{-}2^{+}$ $CD4^{+}$	PD-1, CTLA-4, TIM3, LAG3 HLA-DR
Th2 T cell	$CCR3^{+}$ $CD4^{+}$	PD-1, CTLA-4, TIM3, LAG3 IL-4, IL-13 HLA-DR
Treg	$CD25^{+}$ $CD127^{-}$ $CD4^{+}$	PD-1, CTLA-4, TIM3, LAG3 HLA-DR
Cytotoxic T	$CD8^{+}$ CD27 CD38 CD107a	PD-1, CTLA-4, TIM3, LAG3, CD25, HLA-DR
B Cells		
Mature B	CD19 CD20 IgG IgM	PD-1 CD24 HLA-DR
NK Cells		
NK cells	CD3 CD56 CD335 CD337 CD16 CD158a/h	PD-1 CD2

Cell Name	Identifying Markers	Variable Markers
	CD158b	
Myeloid		
Granulocytes/ GrMDSCs	$CD14^{-}$ $CD15^{+}$ $CD16^{+}$ $CD33^{+/low}$ $CXCR2^{+}$	PD-1 PD-L1 HLA-DR IDO
Monocytes/ MoMDSCs	$CD14^{+}$ $CD15^{-}$ $CD16^{+}$ $CD33^{+}$ $CCR2^{+}$	PD-1 PD-L1 HLA-DR IDO
M1 macrophage	$CD11b^{+}$ $CD86^{+}$ $CD68^{+}$ $CD80^{+}$	PD-1 PD-L1 HLA-DR IDO
M2 macrophage	$CD11b^{+}$ $CD68^{+}$ $CD163^{+}$ $CD206^{+}$	PD-1 PD-L1 HLA-DR IDO
Dendritic cells	$CD11b^{+}$ $CD11c^{+}$ CD103 CD80 CD86	PD-1 PD-L1 HLA-DR IDO

Antibodies for IHC-P

Name	Clone	Target	Host Ig class/subclass	Conjugate
CD4	N1UG0	CD4 T cells	Mouse IgG2b	Unconjugated
CD8	C8/144B	CD8 T cells	Mouse IgG1	DyLight 550
CD45	HI30	Hematopoitic Lineages	Mouse IgG1	Alexa Fluor 488
CD279/PD-1	EH33	Activated/Exausted T Cells	Mouse IgG2a	Unconjugated
CD152/CTLA-4	Ipilimumab	Activated/Exausted T Cells	human	Unconjugated
FOXP3	450	Regulatory T cells	Mouse IgG3	Unconjugated
CD183/CXCR3	polyclonal	Inflammatory T cells	rabbit	unconjugated

Secondary antibodies for IHC-P

Name	Clone	Target	Host Ig class/subclass	Conjugate
Goat anti-human IgG	Polyclonal	Human	Goat IgG	DyLight 405

Name	**Clone**	**Target**	**Host Ig class/subclass**	**Conjugate**
Goat anti-rabbit IgG	Polyclonal	Rabbit IgG	Goat IgG	Alexa Fluor 430
Goat anti-mouse IgG3	Polyclonal	Mouse IgG3	Goat IgG	Alexa Fluor 594
Goat anti-mouse IgG2a	Polyclonal	Mouse IgG2a	Goat IgG	Alexa Fluor 647
Goat anti-mouse IgG2b	Polyclonal	Mouse IgG2b	Goat IgG	IRDye 680LT

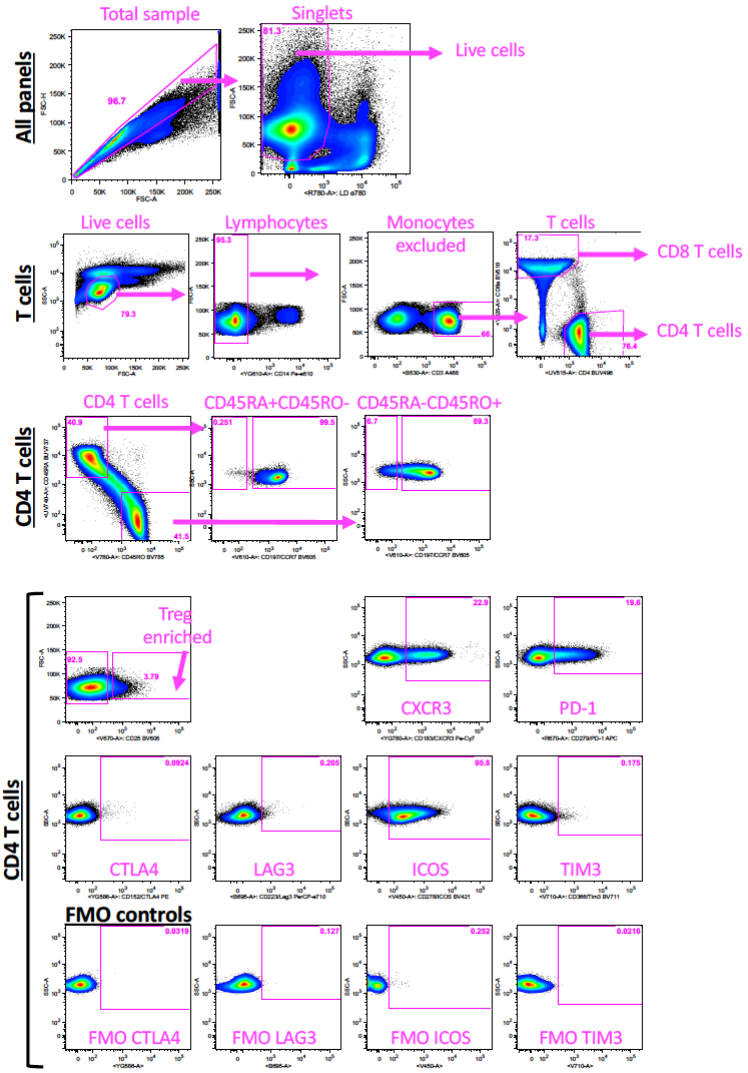


Figure 3: Gating hierarchy to identify lymphocyte subsets.

Healthy donor peripheral blood isolated using BD CPT tubes is shown. Top row: Initial gating strategy applied to all samples and stains. Singlets were selected from total samples, and live cells were selected based on dim fluorescence of amine-reactive dye. The remaining gating strategy for CD4 T cells is shown through progressive sub-gating. Third row shows CD45RA+CD45RO-: naïve (CCR7+) and effector (CCR7-) T cells followed by CD45RA-CD45Ro+: central memory (CCR7+) and effector memory (CCR7-) T cells. Rows 4-6 show expression of individual markers within total CD4 T cells along with fluorescence minus one (FMO) controls used to define positive events where appropriate. Gate combinations are applied in a Boolean fashion. For example, the seven gates drawn using seven markers shown for CD4 T cells results in 2^7 potential expression pattern combinations (128 gates applied to CD4 T cells). Gating strategy is repeated for CD8 T cells