STUDY NUMBER: CASE 1317

ClinicalTrials.gov NCT #: NCT 03452579

Version Date: Protocol Date 10/05/2017
Protocol Date 10/24/2017
Protocol Date Amendment 1 05/03/2018
Protocol Date Amendment 2 05/21/2018
Protocol Date Amendment 3 01/06/2020
Protocol Date Amendment 4 03/20/2020
Protocol Date Amendment 5 07/14/2021

STUDY TITLE: CA209-382 A Randomized Phase 2 Open Label Study of Nivolumab plus standard dose Bevacizumab versus Nivolumab plus low dose Bevacizumab in Recurrent Glioblastoma (GBM)

PRINCIPAL INVESTIGATOR: David Peereboom M.D.
Case Comprehensive Cancer Center
Cleveland Clinic
Taussig Cancer Center
9500 Euclid Avenue, CA-51
Cleveland, OH 44195
[redacted]

CO-PI: David A. Reardon, M.D.
Center for Neuro-Oncology
Dana-Farber Cancer Institute
450 Brookline Avenue, D2134
[redacted]

[redacted]

STATISTICIAN: [redacted]

STUDY COORDINATOR: [redacted]

SPONSOR: Case Comprehensive Cancer Center

SUPPORT/FUNDING: Cleveland Clinic/Bristol Myers Squibb

SUPPLIED AGENT**:** Nivolumab

IND #: [redacted]

OTHER AGENT**:** Bevacizumab

SUMMARY OF CHANGES

Protocol Date	Section	Change
10/05/2017		Initial IRB approval
10/24/2017	Table 9.2-1	Table 9.2-1 Correlative Study information inadvertently omitted from table 9.2-1 Cytokine/chemokine/PBMC Correlative studies and Tumor Tissue added to screening assessments
10/24/2017	Page 6	Removal of the word monotherapy
10/24/2017	Page 28	Corrected nivolumab monotherapy to state nivolumab + standand bevacizumab
10/24/2017	Page 39 6.5.1	Corrected nivolumab to state nivolumab plus standard bevacizumab
10/24/2017	Page 39 6.5.1 1-1	Corrected nivolumab monotherapy to state Nivolumab (BMS-936558) plus standard bevacizumab
05/03/2018	Page 1	NCT # added, Updated protocol version date added, updated statistician contact information
05/03/2018	Page 16	Appendices updated to include 7.0 NANO Scale
05/03/2018	Page 30	Information required for subject randomization updated
05/03/2018	Page 30	Patients name updated to Patients Initials, Patient Medical Record # updated to Patient ID
05/03/2018	Page 32	® added after Mirena
05/03/2018	Page 33 section 4.2	Exclusion Criteria 1 updated to “more than one recurrences of GBM”
05/03/2018	Page 36	Typo of sequelae corrected

Protocol Date	Section	Change
05/03/2018	Page 41 Section 6.5.1	Drug names of nivolumab and bevacizumab updated from upper case to lower case
05/03/2018	Page 41	Time of infusion changed from 30 minutes after completion of the nivolumab infusion to 10 minutes
05/03/2018	Page 63	Injection solution typo corrected
05/03/2018	Page 68 Table 9.2-1	MGMT added, Tumor biopsy changed to tumor tissue, notes for tumor tissue details amended, oxygen saturation–by pulse oximetry. Pulse oximetry at rest and after exertion deleted, physical measurements of Karnofsky performance score changed to KPS, laboratory tests amended-serum urea level deleted, B/C(HBVsAG, HCV antibody deleted, HIV,HepVsAg,HCV added
05/03/2018	Page 70 Table 9.2-2	Window updated to 3-4 days, Physical exam amended to day 1 of each cycle +/-4 days, vital signs and oxygen saturation amended, clarification regarding C1D1 labs added, clarification of labs after C1 D1 added
05/03/2018	Page 71 Table 9.2-2	Cytokine/chemokine/PBMC Correlative Studies added to on study assessments. To be obtained at Week 4, Week 8, then at every MRI visit until progression
05/03/2018	Page 71 Table 9.2-3	Deletion of Oxygen saturation by pulse oximetry. Pulse oximetry at rest and after exertion
05/03/2018	Pages 75-81	Statistical Section of the protocol amended Section 12.1 Amended Section 12.2.5 Amended
05/03/2018	Page 115	Appendix 7 Cytokine/chemokine/PBMC Correlative Studies amended to include details of tissue slides, location of tissue submission, blood collection details clarified
05/03/2018	Page 118	Appendix 9 NANO Scale added
05/03/2018		CTCAE version updated to CTCAE v 5.0 throughout protocol
05/03/2018	Pages 7,26,27 ,31,74	First or second recurrence amended to state only first recurrence

Protocol Date	Section	Change
05/03/2018	Page 34 4.2	Exclusion criteria amended to ≥ NCI CTCAE Grade 3 within 6 months prior to start of study treatment
05/21/2018	Inclusion criteria d	First or second recurrence amended to state only first recurrence
05/21/2018	Inclusion criteria g	Deleted
01/06/2020	Cover page and Protocol footer	Updated to include Amendment number and version date
01/06/2020	All Protocol	Patients amended to Subjects throughout protocol
01/06/202	Study Design	Deleted "may have up to 2 recurrences" amended to state "Subjects must have received previous treatment with radiotherapy and one recurrence"
01/06/2020	3.2 Number of Subjects	Arm B (nivolumab + reduced dose bevacizumab) amended to Arm B (nivolumab + low dose bevacizumab)
01/06/2020	3.3 Study Phases	Arm B (nivolumab + reduced dose bevacizumab) amended to Arm B (nivolumab + low dose bevacizumab) The second infusion will be bevacizumab, and will start no sooner than 30 minutes amended to no sooner than 10 minutes All of the laboratory tests and vital signs will be collected prior to study drug dosing at the time points specified in Section amended from 10.0 to 9.2 Study drug dosing may be delayed for toxicity. See Section 7 amended to 6.6
01/06/2020	3.4	After verifying each patient's eligibility status and administering informed consent, the patient will be enrolled into the study by the study coordinator to obtain the subject number amended to study coordinator "or research nurse" Added sentence -If the subject withdraws or screen fails before starting treatment, the assigned subject number will not be re-issued Surgery type amended to complete resection, near complete resection or biopsy
01/06/2020	4.0	Gender,Age,Race deleted

Protocol Date	Section	Change
01/06/2020	4.1	Applies to covered entities in the USA only deleted Addition of bullet point i) Up to ten unstained slides of 5 microns thickness or a block of tissue will be required to be sent if tissue is available. If the tissue is not available then Principal investigator permissions is required prior to enrollment Bullet point d) and e) amended. Bullet point g) and h) deleted Deleted-Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence Section Women of Child Bearing Potential (WOCBP) amended
01/06/2020	4.2	Bullet point h) amended from (defined as systolic blood pressure ≥150 mmHg and /or diastolic blood pressure ≥100 mmHg) to (defined as systolic blood pressure ≥160 mmHg and /or diastolic blood pressure ≥100 mmHg) Bullet point l) amended from ≥ NCI CTCAE Grade 3 within 6 months to within 3 months
01/06/2020	4.4	Section Deleted
01/06/2020	4.5.2	Appendix 6 amended to added hepatoxicity article
01/06/2020	5.0	Language added for studies using the Forte EDC (Overture) for data collection
01/06/2020	6.5.1	Arm B amended to state Arm B(nivolumab plus low dose bevacizumab) Table 6.5.1-2: "in combination" deleted and replaced with "plus low dose" Nivolumab will be given every two weeks at a dose of 3mg/kg amended to 240mg
01/06/2020	6.5.2	The dose of bevacizumab will be based on with at study entry amended to based on weight at screening. "guidelines" amended to "or per institutional guidelines."
01/06/2020	6.5.3	Section 7 amended to Section 6.8
01/06/2020	7.4	Language added to clarify that all SAEs are to be reported to Sponsor-Investigator Manmeet Ahluwalia M.D.
01/06/2020	Table 8	Venous Thrombosis. If the planned duration of full-dose anticoagulation amended from >2 weeks to ≥ 2 weeks

Protocol Date	Section	Change
01/06/2020	9.1.2	Amended Arm B form reduced dose bevacizumab to low dose bevacizumab
01/06/2020	9.2.1	Amended Study Calendar, deleted HIV, footnote bb added, footnote q will remain blank
01/06/2020	15.0	Appendix numbers amended.
01/06/2020	Appendix 6	Hepatotoxicity article added
01/06/2020	Appendix 10	Appendix added to include corrected NANO scale
01/06/2020	All Protocol	Updated protocol where is states reduced dose bevacizumab to low dose bevacizumab for consistency
01/06/2020	Table of Contents	Corrected numbering of section 12.0
03/20/2020	Cover page and Protocol footer	Updated to include Amendment number and version date
03/20/2020	Section 9.1.2	Added: In view of the Covid 19 crisis, all in person visits can be substituted for virtual visit. All nursing toxicity checks can be performed over the phone rather than in person
03/20/2020	Section 9.2 Study Calendar	cc superscript added to Physical & Neurological exam, vital signs, Performance Status (KPS)
03/20/2020	Section 9.2 Study Calendar	cc superscript details added: in view of covid-19 crisis, virtual visits will be allowed and the need for physical exam as long as patients is asymptomatic will be waived
07/14/2021	Cover page	Updated Sponsor-Investigator to David Peereboom M.D.
07/14/2021	Cover page	Updated Statistician to Wei Wei (Austin)
07/14/2021	Section 5.0	Updated EDC language to The Advarra EDC™ and OnCore™ databases
07/14/2021	7.8	Updated Sponsor-Investigator to David Peereboom M.D.
07/14/2021	Section 11.1	Updated EDC language to The Advarra EDC™ and OnCore™ databases

Protocol Date	Section	Change
07/14/2021	Section 9.2	dd superscript details added to tumor assessments: patients who remain on study after 3 years, MRIs to be done every 12 weeks (+/- 1 week)
07/14/2021	Appendix 8	Updated Sponsor-Investigator to David Peereboom M.D.

STUDY SCHEMA

SYNPOSIS

Background

The outcome for glioblastoma (GBM) remains dismal with a median survival of approximately 15 months and nearly all cases recur despite progress in surgical techniques, radiation and chemotherapies[1,2]. A number of single arm phase II studies using immunotherapy approaches in subjects with GBM provide support for considering an immunotherapy[3-7] particularly a strategy designed to reverse cancer-mediated immune suppression[8]. T cells have access to antigens within the CNS, and the blood brain barrier does not form an absolute barrier to immune responses; the concept of "immunologic privilege" in the CNS has been refuted[9]. Subjects with GBM have a variety of mechanisms that contribute to an overall state of immune suppression. Primed CD8+ cytotoxic T cells gain CNS access, however, the cells are functionally impaired as evidenced by the lack of tumor eradication. Ex vivo studies demonstrate a lack of effector/activated T cells in the glioma microenvironment[10]. Gliomas secrete factors such as prostaglandin E2 and TGF β that are capable of suppressing cytotoxic responses of T cells against tumor targets. Co-stimulatory inhibitory molecules like B7-H1 are expressed in malignant gliomas and can further inhibit immune responses[11]. T-regulatory cells, which suppress effector T-cell responses, are increased in the peripheral circulation and within the tumors of subjects with glioma[12,13] and more profound immunosuppression is associated with worse outcome[14].

Immune checkpoint blockade is a rapidly advancing therapeutic approach in the field of immuno-oncology and treatment with investigational agents targeting this mechanism has induced regressions in several types of cancer. The programmed death 1 (PD-1) receptor is an important cellular target that play a key role in regulating adaptive immunity. Glioblastoma is an aggressive brain tumor with high mortality and morbidity despite current treatments. The significant unmet clinical need for subjects and preclinical data suggesting involvement of immunologic factors in GBM disease course support the investigation of checkpoint inhibitors for therapeutic potential. Nivolumab monotherapy has demonstrated clinical activity across several tumor types, including advanced melanoma, NSCLC, and RCC. Nivolumab has demonstrated a manageable safety profile in subjects > 700 subjects across all clinical trials. The most common AEs included fatigue, rash, pruritus, diarrhea, and nausea.

Bevacizumab (Avastin; Genentech, South San Francisco, CA) is a humanized monoclonal antibody that inhibits VEGF and is the first antiangiogenic therapy to be approved for use in subjects with cancer. The BRAIN study, a phase II randomized trial evaluated the role of

bevacizumab (alone or in combination with irinotecan) in 167 subjects with recurrent GBM. The progression free survival (PFS) at 6 months (PFS-6) was 42.6% and 50.3%, objective response rate (ORR) was 28.2% and 37.8% and median overall survival (OS) was 9.2 months and 8.7 months in the monotherapy and combination arms respectively. In a study done at the National Cancer Institute (NCI), 48 subjects with recurrent GBM were treated with bevacizumab producing a response rate (RR) of 35%, PFS-6 of 29% and a median OS of 31 weeks. Clinical benefit was also evident with decreasing cerebral edema, tapering steroid doses and improvement in neurological function in nearly half of the subjects. Nonetheless, the optimal dose of bevacizumab for GBM subjects remains unclear. A recent retrospective analysis of 219 subjects confirmed that lower dosing was associated with enhanced survival.[15] In this study, subjects treated with < 10 mg/kg every other week of bevacizumab had a median OS of 9 months compared to only 5 months for those treated with standard 10 mg/kg biweekly (p=0.001). Similar improved survival benefit associated with lower bevacizumab dosing was confirmed in a validation cohort (n=109 subjects). The exact mechanism of improved survival is unclear but standard bevacizumab dosing can significantly decrease perfusion and tumor vasculature permeability, leading to intratumoral hypoxia which may in turn drive GBM invasion and infiltration.[16-20] In contrast, lower doses of anti-angiogenic agents can normalize tumor vasculature leading to enhanced intratumoral immune cell infiltration.[21,22]

The rationale for combining nivolumab with bevacizumab includes increasing data demonstrating that VEGF inhibition can enhance the anti-tumor benefit of immunotherapies by decreasing immunosuppression, enhancing dendritic cell and T cell activity and decreasing Treg activity.[23-25],[26-29] Enhanced anti-tumor immune responses have also been demonstrated in preclinical models following VEGF blockade[22,24-35] and a clinical trial has recently demonstrated that bevacizumab improved therapeutic outcome of CTLA-4 blockade among metastatic melanoma subjects.[36]

The current study will evaluate the anti-tumor activity as well as safety of nivolumab in combination with bevacizumab administered according to standard and reduced dosage schedules for recurrent glioblastoma subjects. Although the hypothesis is that VEGF blockade will enhance the anti-tumor activity of anti-PD-1/PD-L1 therapy, a currently unanswered critical question is whether there is an optimized dosing schedule of bevacizumab to do so. Standard bevacizumab dosing, administered as single agent therapy, represents the approved schedule for recurrent GBM based on durable radiographic responses.[37] In fact, as a monotherapeutic, a higher dosing schedule of bevacizumab may be required to achieve a sufficient anti-angiogenic effect to translate into anti-tumor benefit. However, higher (standard) dosing has also been shown to significantly decrease perfusion and tumor vasculature permeability, leading to intratumoral hypoxia which may in turn drive GBM invasion and infiltration.[16,17,38-40] In contrast, a lower dosing schedule has been shown to

normalize rather than eradicate tumor vasculature, leading to improved blood flow, less hypoxia and enhanced delivery of co-administered anti-tumor agents in preclinical cancer models including GBM.[41,42] Normalized vasculature has also been associated with enhanced survival among cancer subjects including those with GBM treated with anti-VEGF therapy.[43,44] Furthermore, additional preclinical studies demonstrate that normalized tumor vasculature following reduced anti-VEGF therapy also leads to enhanced intratumoral immune cell infiltration.[22,45] Finally, a recently published preclinical study evaluating lower versus higher doses of anti-VEGF therapy showed that only lower anti-VEGF therapy dosing led to enhanced immune infiltrate and improved survival following co-administration with an anti-tumor immunotherapeutic.[22]

A prospective evaluation of standard versus reduced bevacizumab dosing has not been conducted for subjects with GBM. However, a retrospective review of 219 subjects treated with bevacizumab showed that subjects treated with lower dose intensity (< 5 mg/kg per week) of bevacizumab had longer PFS and OS when compared with those treated with the standard 10 mg/kg biweekly dosing. [46]

Investigational Product(s), Dose and Mode of Administration, Duration of Treatment with Investigational Product(s):

- Nivolumab (BMS-936558) administered IV over 30 minutes at 240 mg flat dose and bevacizumab administered IV at 10 mg/kg every 2 weeks until progression (Arm A)
- Nivolumab (BMS-936558) administered IV over 30 minutes at 240 mg flat dose and bevacizumab administered IV at 3 mg/kg every 2 weeks until progression (Arm B)

Study design

This is a randomized, open-label, phase 2 safety study of nivolumab and bevacizumab administered according to standard and low dosage schedules in adult (≥ 18 years) subjects with a first recurrence of glioblastoma (GBM). Subjects must have received previous treatment with radiotherapy and one recurrence. The study will allow subjects that require decadron up to 4 mg/ day to participate.

PROTOCOL SUMMARY

Protocol Number/Title	Case 1317/ CA209-382 A Randomized Phase 2 Open Label Study of Nivolumab plus standard dose Bevacizumab versus Nivolumab plus low dose Bevacizumab in Recurrent Glioblastoma (GBM)
Study Phase	Phase 2
Brief Background/Rationale	The outcome for recurrent glioblastoma (GBM) remains dismal despite progress in surgical techniques, radiation and chemotherapies[1,2]. A number of single arm phase II studies using immunotherapy approaches in subjects with GBM provide support for considering an immunotherapy[3-7] particularly a strategy designed to reverse the prominent cancer-mediated immune suppression[8]. T cells have access to antigens within the CNS, and the blood brain barrier does not form an absolute barrier to immune responses; the concept of "immunologic privilege" in the CNS has been refuted[9]. Subjects with GBM have a variety of mechanisms that contribute to an overall state of immune suppression.

	Primed CD8+ cytotoxic T cells gain CNS access, however, the cells are functionally impaired as evidenced by the lack of tumor eradication. Ex vivo studies demonstrate a lack of effector/activated T cells in the glioma microenvironment[10]. Gliomas secrete factors such as prostaglandin E2 and TGF β that are capable of suppressing cytotoxic responses of T cells against tumor targets. Co-stimulatory inhibitory molecules like B7-H1 are expressed in malignant gliomas and can further inhibit immune responses[11]. T-regulatory cells, which suppress effector T-cell responses, are increased in the peripheral circulation and within the tumors of subjects with glioma[12,13] and the subjects with more profound immunosuppression is associated with worse outcome[14]. Immune checkpoint blockade is a rapidly advancing therapeutic approach in the field of immuno-oncology and treatment with investigational agents targeting this mechanism has induced regressions in several types of cancer. Cytotoxic T-lymphocyte–associated antigen 4(CTLA-4) and programmed death 1 (PD-1) receptor are two important cellular targets that play complementary roles in regulating adaptive immunity. Whereas PD-1 contributes to T-cell exhaustion in peripheral tissues, CTLA-4 inhibits at earlier points in T-cell activation[63]. Nivolumab (BMS-936558; anti-PD-1 monoclonal antibody) is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands promote immune responses and antigen-specific T cell responses to both foreign and self-antigens. PD-1 receptor blockade by nivolumab is a new approach for immunotherapy of tumors. Nivolumab monotherapy has demonstrated clinical activity across several tumor types, including advanced melanoma, NSCLC, and RCC.

	Increasing data suggests that VEGF inhibition can enhance the anti-tumor benefit of immunotherapies. First, VEGF is known to significantly contribute to the immunosuppressive ability of tumors.[23-25] Specifically, VEGF can inhibit dendritic cell maturation and antigen presentation, induce apoptosis of CD8+ T cells, enhance Treg activity and diminish infiltration of T cells across tumor endothelium.[26-29] Second, preclinical studies demonstrate that immunotherapeutics may be combined with VEGF inhibitors to generate enhanced anti-tumor benefit.[22,24-35] Specifically, VEGF inhibition can diminish immunosuppressive features of tumors[26-29,32,34,35] and enhance the anti-tumor activity of immunotherapies.[30-32,34,35] Third, preclinical strategies to normalize tumor vasculature, including administration of ant-VEGF therapy, can shift tumor-associated macrophages from an M2 immune-inhibitory phenotype to an immune-stimulatory M1-phenotype, as well as increase tumor infiltrating CD8+ T cells and enhance survival following whole tumor cell vaccination.[22] Finally, data from a recently published phase I study among metastatic melanoma subjects revealed that administration of bevacizumab with ipilimumab, an inhibitor of the CTLA-4 immune checkpoint, led to improved overall survival as well as increased immune cell trafficking into tumor sites.[36] The rationale for evaluating two dose levels of bevacizumab in combination with standard nivolumab dosing is based on several factors. First, although a lower bevacizumab dose schedule has not been prospectively evaluated among recurrent glioblastoma subjects, a recent retrospective analysis of 219 subjects confirmed that lower dosing was associated with enhanced survival.[15] In this study, subjects treated with < 10 mg/kg every other week of bevacizumab had a median OS of 9 months compared to only 5 months for those treated with standard 10 mg/kg biweekly (p=0.001). Similar improved survival benefit associated with lower bevacizumab dosing was confirmed

	in a validation cohort (n=109 subjects). Second, although standard bevacizumab dosing, administered as single agent therapy at 10 mg/kg every 2 weeks, represents the approved schedule for recurrent GBM based on durable radiographic responses[37] higher (standard) dosing has also been shown to significantly decrease perfusion and tumor vasculature permeability, leading to intratumoral hypoxia which may in turn drive GBM invasion and infiltration.[16-20] In contrast, a lower dosing schedule has been shown to normalize rather than eradicate tumor vasculature, leading to improved blood flow, less hypoxia and enhanced delivery of co-administered anti-tumor agents in preclinical cancer models including GBM.[41,42] Normalized vasculature has also been associated with enhanced survival among cancer subjects including those with GBM treated with anti-VEGF therapy.[43,64] Furthermore, additional preclinical studies demonstrate that normalized tumor vasculature following reduced anti-VEGF therapy also leads to enhanced intratumoral immune cell infiltration.[21,22] Finally, a recently published preclinical study evaluating lower versus higher doses of anti-VEGF therapy showed that only lower anti-VEGF therapy dosing led to enhanced immune infiltrate and improved survival following co-administration with an anti-tumor immunotherapeutic.[22] In addition to these potential advantages, bevacizumab at either standard or reduced dosing, is expected to decrease tumor vessel permeability[62] which may lessen the cerebral edema that typically accompanies GBM recurrence. By decreasing cerebral edema, bevacizumab may reduce the need for systemic corticosteroids such as dexamethasone which are routinely used to treat symptomatic cerebral edema but which may also abrogate anti-tumor immunoreactivity generated by PD-1 blockade.
Primary Objective	Primary Endpoint(s) To evaluate the efficacy of nivolumab when administered with standard and reduced bevacizumab dosing among

	recurrent glioblastoma subjects as measured by the rate of overall survival at twelve months (OS-12).
Secondary Objective(s)	Secondary Endpoint(s) To evaluate the safety and tolerability of nivolumab in combination with bevacizumab administered according to standard and reduced dosage schedules for recurrent glioblastoma subjects. To compare progression free survival (PFS) at 6 months of nivolumab when administered with standard and reduced bevacizumab dosing for recurrent glioblastoma subjects. To compare the overall survival rate of nivolumab when administered with standard and reduced bevacizumab dosing for recurrent glioblastoma. To compare progression free survival (PFS) of when administered with standard and reduced bevacizumab dosing for recurrent glioblastoma subjects. To compare the objective response rate (ORR) of nivolumab and bevacizumab administered according to standard and reduced dosage schedules for recurrent glioblastoma subjects
Exploratory Objective(s)	Exploratory Endpoints (s) To evaluate whether baseline values or subsequent changes in circulating immunologic parameters (including but not limited to the number of T, B and NK cells; the number of T cell subsets; soluble circulating cytokines) are associated with outcome. To assess neurologic functioning in the treatment arms using the Neurologic Assessment in Neuro-Oncology (NANO). To assess the perfusion and diffusion base imaging to correlate with changes and response to nivolumab when administered with standard and reduced bevacizumab

	dosing. To assess response using the immunotherapy response assessment in neuro-oncology criteria relative to survival.

ABBREVIATIONS

CCCC	Case Comprehensive Cancer Center
CRF	Case Report Form
DCRU	Dahm's Clinical Research Unit
DSTC	Data Safety and Toxicity Committee
FDA	Food and Drug Administration
ICF	Informed Consent Form
IRB	Institutional Review Board
PRMC	Protocol Review and Monitoring Committee
SOC	Standard of Care
CCF	Cleveland Clinic Foundation
UH	University Hospitals

TABLE OF CONTENTS

15. APPENDICES

1.0 Karnofsy and ECOG Performance Status Scales
2.0 Immunotherapy response assessment in neuro-oncology (IRANO) criteria
3.0 Sample of drug ordering and pharmacy reference manual
4.0 Adverse event reporting
5.0 Recommended MRI sequences
6.0 Hepatoxicity Article
7.0 AE Management Algorithms
8.0 Correlative Studies
9.0 Laboratory Requisition
10.0 NANO Scale

1.0 Introduction

1.1 Background of Study Disease

Glioblastoma (GBM)

The outcome for glioblastoma (GBM) remains dismal with a median survival of approximately 15 months and nearly all cases recur despite progress in surgical techniques, radiation and chemotherapies[1,2]. A number of single arm phase II studies using immunotherapy approaches in subjects with GBM provide support for considering an immunotherapy [3-7]particularly a strategy designed to reverse the prominent cancer-mediated immune suppression[8]. T cells have access to antigens within the CNS, and the blood brain barrier does not form an absolute barrier to immune responses; thus, the concept of "immunologic privilege" in the CNS has been refuted [9]. Subjects with GBM have a variety of mechanisms that contribute to an overall state of immune suppression. Primed CD8+ cytotoxic T cells gain CNS access, however, the cells are functionally impaired as evidenced by the lack of tumor eradication. Ex vivo studies demonstrate a lack of effector/activated T cells in the glioma microenvironment [10]. Gliomas secrete factors such as prostaglandin E2 and TGF β that are capable of suppressing cytotoxic responses of T cells against tumor targets. Co-stimulatory inhibitory molecules like B7-H1 are expressed in malignant gliomas and can further inhibit immune responses[11]. T-regulatory cells, which suppress effector T-cell responses, are increased in the peripheral circulation and within the tumors of subjects with glioma [12,13], and the subjects with more profound immunosuppression is associated with worse outcome[14]

1.2 Name and Description of Investigational Agent

Nivolumab

Immune checkpoint blockade is a rapidly advancing therapeutic approach in the field of immuno-oncology and treatment with investigational agents targeting this mechanism has induced regressions in several types of cancer. Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) receptor are two important cellular targets that play complementary roles in regulating adaptive immunity. Whereas PD-1 contributes to T-cell exhaustion in peripheral tissues, CTLA-4 inhibits at earlier points in T-cell activation [Curran 2010).

Nivolumab (BMS-936558; anti-PD-1 monoclonal antibody) is a fully human monoclonal

immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands promote immune responses and antigen-specific T cell responses to both foreign and self-antigens. PD-1 receptor blockade by nivolumab is a new approach for immunotherapy of tumors. Nivolumab monotherapy has demonstrated clinical activity across several tumor types, including advanced melanoma, NSCLC, and RCC. Nivolumab has demonstrated a manageable safety profile in subjects > 700 subjects across all clinical trials. The most common AEs included fatigue, rash, pruritus, diarrhea, and nausea.
Nivolumab monotherapy is currently being studied in phase 3 clinical trials in advanced melanoma, renal cell carcinoma (RCC) and non-small cell lung carcinoma (NSCLC).

1.2.1 Preclinical Data

Pharmacology

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

Based upon the mechanistic rationale discussed above and promising results from a preliminary clinical study (CA209004) using the combination of nivolumab and ipilimumab in subjects with un-resectable or metastatic melanoma, the safety and efficacy of nivolumab as a single agent or in combination with ipilimumab in subjects with recurrent GBM was evaluated in a clinical trial that has completed accrual and the final results are awaited (NCT02017717).

1.2.2 Clinical Data

Although the efficacy of check point inhibitors such as nivolumab have not previously been studied in GBM, a multicenter Phase 2 study to evaluate the response of brain metastases to ipilimumab was previously performed (CA184042). Subjects (N = 71) with advanced Stage IV

melanoma and measureable active brain metastases were randomized to ipilimumab monotherapy. The study demonstrated that ipilimumab had clinical activity in subjects with melanoma brain metastases - with some subjects showing prolonged clinical responses, disease control, and prolonged survival. Ipilimumab did not cause unexpected neurological toxicity in subjects with brain metastases. There is a large ongoing study of Randomized Phase 3 Open Label Study of Nivolumab versus Bevacizumab and a Safety Study of Nivolumab or Nivolumab in Combination with Ipilimumab in Adult Subjects with Recurrent Glioblastoma (NCT 02017717).

1.2.3 Clinical Pharmacokinetics of Nivolumab

- **Safety Pharmacology**

The overall safety experience with nivolumab, as a monotherapy or in combination with other therapeutics, is based on experience in approximately 4,000 subjects treated to date. For monotherapy, the safety profile is similar across tumor types. The only exception is pulmonary inflammation adverse events (AEs), which may be numerically greater in subjects with NSCLC, because in some cases, it can be difficult to distinguish between nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes. There is no pattern in the incidence, severity, or causality of AEs to nivolumab dose level (Reference: INVESTIGATOR BROCHURE Nivolumab, BMS-936558, MDX1106 Version 13, July 2014)

- **Pharmacokinetics and Absorption, Distribution, Metabolism, and Excretion**

The single-dose pharmacokinetics (PK) of nivolumab was linear and dose-proportional in the range of 0.3 mg/kg to 10 mg/kg. The multiple-dose PK of nivolumab was linear with dose-proportional increases in maximum serum concentration (Cmax) and area under the concentration-time curve over the dosing interval (AUC[TAU]) in the range of 0.1 mg/kg to 10 mg/kg. Both elimination and distribution of nivolumab in the dose range studied appear to be independent of dose in the dose-ranging studies, while the end of infusion and minimum serum concentration (Cmin) after the first dose were approximately dose proportional. Based on population PK (PPK) results (preliminary data), clearance of nivolumab is independent of dose in the dose range (0.1 mg/kg to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights and, therefore, is appropriate for future clinical trials with nivolumab.

Single-dose PK of nivolumab was studied in 39 subjects with cancer. The single-dose PK of nivolumab was linear and dose-proportional in the range of 0.3 mg/kg to 10 mg/kg. The mean terminal T-HALF of nivolumab ranged between 17 and 25 days across the dose range of 0.3

mg/kg to 10 mg/kg. Geometric mean total clearance varied from 0.13 mL/h/kg to 0.19 mL/h/kg, while mean volume of distribution varied between 83 mL/kg and 113 mL/kg across doses. The clearance and half-life of nivolumab are consistent with that of IgG4.

The multiple-dose PK of nivolumab given Q2W was determined from MDX1106-03 study as well as by population PK using data from 669 subjects across nivolumab studies.
Multiple-dose PK of nivolumab following Q2W dosing was linear with dose-proportional increase in Cmax and AUC(TAU) in the studied range of 0.1 mg/kg to 10 mg/kg. Nivolumab accumulation with Q2W dosing frequency was in the range of 2.9 to 3.3 based on AUC(TAU), 2.0 to 2.4 based on Cmax, and 3.1 to 4.8 based on Cmin. A PPK model was developed by nonlinear mixed effect modeling using data from 669 subjects.

Nivolumab concentration-time data were well described by a linear, 2-compartment, 0-order IV infusion model with first-order elimination. Nivolumab PK was found to be linear, dose independent, and time invariant. The geometric mean of terminal T-HALF was 25.6 days and the typical clearance was 8.8 mL/h, which are consistent with those of full human immunoglobulin antibodies. Clearance of nivolumab is independent of dose in the dose range (0.1 mg/kg to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights.

Nivolumab monotherapy has been extensively studied in a number of tumor types including NSCLC, MEL, RCC, and CRC with body weight normalized dosing (mg/kg). Nivolumab pharmacokinetics (PK) and exposures of subjects in these studies have been characterized by population pharmacokinetic (PPK) analysis of data collected these studies, together with PK data from several phase 1, 2, and 3 clinical studies of nivolumab monotherapy in solid tumors. Population PK (PPK) analyses have shown that the PK of nivolumab are linear, with dose proportional exposures over a dose range of 0.1 mg/kg to 10 mg/kg, and are similar across tumor types. Nivolumab clearance and volume of distribution were found to increase with increasing body weight, but the increase was less than proportional, indicating that a mg/kg dose represents an over-adjustment for the effect of body weight on nivolumab PK. Given the relationship between nivolumab PK and body weight, a flat dose is expected to lead to lower exposures in heavier subjects, relative to the exposures in lighter subjects.

Using the PPK model, nivolumab steady-state trough, peak and time-averaged concentration (Cminss, Cmaxss, and Cavgss, respectively) were predicted for a flat nivolumab dose of 240 mg Q2W and compared to those following administration of 3 mg/kg Q2W in NSCLC subjects. A dose of 240 mg nivolumab is identical to a dose of 3 mg/kg for subjects weighing 80 kg, which is the approximate median body weight of NSCLC subjects in the 3 Phase 2 and 3 BMS clinical studies of nivolumab monotherapy. The geometric mean values of Cminss,

Cmaxss, and Cavgss with flat dosing are slightly (< 15%) higher than that produced by a 3 mg/kg dose, and the coefficient of variation (cv%) in these measures of exposure are only slightly (< 10%) greater than that of 3 mg/kg dosing.

Across the various tumor types in the BMS clinical program, nivolumab has been shown to be safe and well tolerated up to a dose level of 10 mg/kg, and the relationship between nivolumab exposure produced by 3 mg/kg and efficacy has been found to be relatively flat. Taken together, the PK, safety, and efficacy data indicate that the safety and efficacy profile of 240 mg nivolumab will be similar to that of 3 mg/kg nivolumab.

Thus a flat dose of 240 mg every 2 weeks is recommended for investigation in this study

1.3 Bevacizumab

Bevacizumab is a humanized IgG1 monoclonal antibody (MAb) that binds all biologically active isoforms of human VEGF (or VEGF-A) with high affinity (kd = 1.1 nM). The antibody consists of a human IgG1 framework and the antigen-binding complementarity- determining regions from the murine anti-VEGF MAb A.4.6.1.16-18. Bevacizumab is commercially available and FDA approved for subjects with recurrent glioblastoma. Refer to the package insert for more detailed information on bevacizumab.

1.3.1 Pharmaceutical and Therapeutic Background

VEGF is one of the most potent and specific angiogenic factors, and it has been identified as a crucial regulator of both normal and pathological angiogenesis. VEGF is a secreted, heparin-binding protein that exists in multiple isoforms. Action of VEGF is primarily mediated through binding to the receptor tyrosine kinases VEGFR-1 (Flt-1) and VEGFR- 2 (KDR/Flk-1). The biologic effects of VEGF include endothelial cell mitogenesis and migration, increased vascular permeability, induction of proteinases leading to remodeling of the extracellular matrix, and suppression of dendritic cell maturation. Neutralization of VEGF by A.4.6.1 or bevacizumab has been shown to inhibit the VEGF-induced proliferation of human endothelial cells in vitro and to decrease microvessel density and interstitial pressure in tumor xenografts in vivo.

1.3.2 Preclinical and Clinical Data

The murine parent MAb of bevacizumab, A4.6.1, has demonstrated potent growth inhibition in vivo in a variety of human cancer xenograft and metastasis models, including those for SKLMS-1 leiomyosarcoma, G55 glioblastoma multiforme, A673 rhabdomyosarcoma, Calu-6,

and MCF-7 cell lines.[49-51] The antitumor activity was enhanced with the combination of A4.6.1 and chemotherapeutic agents compared to either agent alone. Furthermore, combined blockage of the VEGF pathway and other growth factor pathways (e.g., EGFR or PDGFR) has also demonstrated additive effects in vivo.[52,53] Associated with the antitumor activity of anti-VEGF MAbs were findings of reduced intratumoral endothelial cells and microcapillary counts as well as reduced vascular permeability and interstitial pressure.

Nonclinical toxicology studies have examined the effects of bevacizumab on female reproductive function, fetal development, and wound healing. Fertility may be impaired in Cynomolgus monkeys administered bevacizumab, which led to reduced uterine weight and endometrial proliferation as well as a decrease in ovarian weight and number of corpora lutea. Bevacizumab is teratogenic in rabbits, with increased frequency of fetal resorption as well as specific gross and skeletal fetal alterations. In juvenile Cynomolgus monkeys with open growth plates, bevacizumab induced epiphyseal dysplasia that was partially reversible upon cessation of therapy. Bevacizumab also delays the rate of wound healing in rabbits, and this effect appeared to be dose dependent and characterized by a reduction of wound tensile strength.

Bevacizumab has been studied in multiple Phase I, Phase II, and Phase III clinical trials and in multiple tumor types. The following discussion summarizes bevacizumab's safety profile and presents some of the efficacy results pertinent to this particular trial. Clinical proof of principle for anti-VEGF therapy with bevacizumab has been provided by the pivotal Phase III trial of bevacizumab (5 mg/kg every 2 weeks) in combination with bolus irinotecan/5-fluorouracil/leucovorin (IFL) in subjects with untreated advanced colorectal cancer (AVF2107g).[54] In that study, the addition of bevacizumab to IFL was associated with an increase in objective responses (45% vs. 35%) and significant prolongations of both time to progression (10.6 vs. 6.2 months) and overall survival (20.3 vs. 15.6 months) compared with IFL.

Based on the survival advantage, bevacizumab was approved in 2004 in the United States for first-line treatment in combination with IV 5-FU-based chemotherapy for subjects with metastatic colorectal cancer. Additional data from Phase III trials in metastatic CRC,[55] non-small cell lung cancer,[56] renal cell carcinoma[57] and metastatic breast cancer[58] have also demonstrated clinical benefit from bevacizumab when added to chemotherapy.

Single agent bevacizumab received accelerated FDA approval in 2009 for recurrent glioblastoma based on favorable results from two Phase II clinical trials.[59,60] In these studies, six-month progression free survival (PFS6) for bevacizumab monotherapy ranged from 29% to 42.6%. Compared to a historical PFS6 of 15% for recurrent GBM,[61] these studies suggest that bevacizumab has significant clinical activity in this patient population.

Nonetheless, the optimal dosing schedule of bevacizumab for glioblastoma subjects remains unknown. Although a lower bevacizumab dose schedule has not been prospectively evaluated among recurrent glioblastoma subjects, a recent retrospective analysis of 219 subjects confirmed that lower dosing was associated with enhanced survival.[15] In this study, subjects treated with < 10 mg/kg every other week of bevacizumab had a median OS of 9 months compared to only 5 months for those treated with standard 10 mg/kg biweekly (p=0.001). Similar improved survival benefit associated with lower bevacizumab dosing was confirmed in a validation cohort (n=109 subjects). The exact mechanism of improved survival is unclear but standard bevacizumab dosing can significantly decrease perfusion and tumor vasculature permeability, leading to intratumoral hypoxia which may in turn drive GBM invasion and infiltration.[16-20] In contrast, lower doses of anti-angiogenic agents can normalize tumor vasculature leading to enhanced intratumoral immune cell infiltration.[21,22]

In addition to these potential advantages, lower bevacizumab dosing is expected to decrease tumor vessel permeability[62] which may lessen the cerebral edema that typically accompanies GBM recurrence. By decreasing cerebral edema, lower dosed bevacizumab may reduce the need for systemic corticosteroids such as dexamethasone which are routinely used to treat symptomatic cerebral edema but which may also abrogate anti-tumor immunoreactivity generated by PD-1 blockade.

1.4 Drug prohibited during the study

Any concurrent drug or other investigational agents for treatment of GBM (i.e., chemotherapy, hormonal therapy, immunotherapy, radiation therapy)

Medications contraindicated with bevacizumab treatment (refer to the package insert, summary of product characteristics (SmPC), or similar document)

1.5 Rationale

Scientific Background

The outcome for recurrent glioblastoma (GBM) remains dismal despite progress in surgical techniques, radiation and chemotherapies[1,2]. A number of single arm phase II studies using immunotherapy approaches in subjects with GBM provide support for considering an immunotherapy[3-7] particularly a strategy designed to reverse the prominent cancer-mediated immune suppression[8]. T cells have access to antigens within the CNS, and the blood brain barrier does not form an absolute barrier to immune responses; the concept of “immunologic

privilege" in the CNS has been refuted[9]. Subjects with GBM have a variety of mechanisms that contribute to an overall state of immune suppression. Primed CD8+ cytotoxic T cells gain CNS access, however, the cells are functionally impaired as evidenced by the lack of tumor eradication. Ex vivo studies demonstrate a lack of effector/activated T cells in the glioma microenvironment[10]. Gliomas secrete factors such as prostaglandin E2 and TGF β that are capable of suppressing cytotoxic responses of T cells against tumor targets. Co-stimulatory inhibitory molecules like B7-H1 are expressed in malignant gliomas and can further inhibit immune responses[11]. T-regulatory cells, which suppress effector T-cell responses, are increased in the peripheral circulation and within the tumors of subjects with glioma[12,13] and the subjects with more profound immunosuppression is associated with worse outcome[14].

Immune checkpoint blockade is a rapidly advancing therapeutic approach in the field of immuno-oncology and treatment with investigational agents targeting this mechanism has induced regressions in several types of cancer. Cytotoxic T-lymphocyte–associated antigen 4(CTLA-4) and programmed death 1 (PD-1) receptor are two important cellular targets that play complementary roles in regulating adaptive immunity. Whereas PD-1 contributes to T-cell exhaustion in peripheral tissues, CTLA-4 inhibits at earlier points in T-cell activation[63].

Nivolumab (BMS-936558; anti-PD-1 monoclonal antibody) is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands promote immune responses and antigen-specific T cell responses to both foreign and self-antigens. PD-1 receptor blockade by nivolumab is a new approach for immunotherapy of tumors. Nivolumab monotherapy has demonstrated clinical activity across several tumor types, including advanced melanoma, NSCLC, and RCC.

Increasing data suggests that VEGF inhibition can enhance the anti-tumor benefit of immunotherapies. First, VEGF is known to significantly contribute to the immunosuppressive ability of tumors.[23-25] Specifically, VEGF can inhibit dendritic cell maturation and antigen presentation, induce apoptosis of CD8+ T cells, enhance Treg activity and diminish infiltration of T cells across tumor endothelium.[26-29] Second, preclinical studies demonstrate that immunotherapeutics may be combined with VEGF inhibitors to generate enhanced anti-tumor benefit.[22,24-35] Specifically, VEGF inhibition can diminish immunosuppressive features of tumors[26-29,32,34,35] and enhance the anti-tumor activity of immunotherapies.[30-32,34,35] Third, preclinical strategies to normalize tumor vasculature, including administration of ant-VEGF therapy, can shift tumor-associated macrophages from an M2 immune-inhibitory phenotype to an immune-stimulatory M1-phenotype, as well as increase tumor infiltrating CD8+ T cells and

enhance survival following whole tumor cell vaccination.[22] Finally, data from a recently published phase I study among metastatic melanoma subjects revealed that administration of bevacizumab with ipilimumab, an inhibitor of the CTLA-4 immune checkpoint, led to improved overall survival as well as increased immune cell trafficking into tumor sites.[36]

The rationale for evaluating two dose levels of bevacizumab in combination with standard nivolumab dosing is based on several factors. First, although a lower bevacizumab dose schedule has not been prospectively evaluated among recurrent glioblastoma subjects, a recent retrospective analysis of 219 subjects confirmed that lower dosing was associated with enhanced survival.[15] In this study, subjects treated with < 10 mg/kg every other week of bevacizumab had a median OS of 9 months compared to only 5 months for those treated with standard 10 mg/kg biweekly (p=0.001). Similar improved survival benefit associated with lower bevacizumab dosing was confirmed in a validation cohort (n=109 subjects).

Second, although standard bevacizumab dosing, administered as single agent therapy at 10 mg/kg every 2 weeks, represents the approved schedule for recurrent GBM based on durable radiographic responses[37] higher (standard) dosing has also been shown to significantly decrease perfusion and tumor vasculature permeability, leading to intratumoral hypoxia which may in turn drive GBM invasion and infiltration.[16-20] In contrast, a lower dosing schedule has been shown to normalize rather than eradicate tumor vasculature, leading to improved blood flow, less hypoxia and enhanced delivery of co-administered anti-tumor agents in preclinical cancer models including GBM.[41,42] Normalized vasculature has also been associated with enhanced survival among cancer subjects including those with GBM treated with anti-VEGF therapy.[43,64] Furthermore, additional preclinical studies demonstrate that normalized tumor vasculature following reduced anti-VEGF therapy also leads to enhanced intratumoral immune cell infiltration.[21,22] Finally, a recently published preclinical study evaluating lower versus higher doses of anti-VEGF therapy showed that only lower anti-VEGF therapy dosing led to enhanced immune infiltrate and improved survival following co-administration with an anti-tumor immunotherapeutic.[22]

In addition to these potential advantages, bevacizumab at either standard or reduced dosing, is expected to decrease tumor vessel permeability[62] which may lessen the cerebral edema that typically accompanies GBM recurrence. By decreasing cerebral edema, bevacizumab may reduce the need for systemic corticosteroids such as dexamethasone which are routinely used to treat symptomatic cerebral edema but which may also abrogate anti-tumor immunoreactivity generated by PD-1 blockade.

2.0 Objectives

2.1 Primary Objective

- To evaluate the efficacy of nivolumab when administered with standard and reduced bevacizumab dosing among recurrent glioblastoma subjects as measured by the rate of overall survival at twelve months (OS-12).

2.2 Secondary Objective(s)

- To evaluate the safety and tolerability of nivolumab in combination with bevacizumab administered according to standard and reduced dosage schedules for recurrent glioblastoma subjects.
- To compare progression free survival (PFS) at 6 months of nivolumab when administered with standard and reduced bevacizumab dosing for recurrent glioblastoma subjects.
- To compare the overall survival rate of nivolumab when administered with standard and reduced bevacizumab dosing for recurrent glioblastoma subjects.
- To compare progression free survival (PFS) of when administered with standard and reduced bevacizumab dosing for recurrent glioblastoma subjects.
- To compare the objective response rate (ORR) of nivolumab and bevacizumab administered according to standard and reduced dosage schedules for recurrent glioblastoma subjects.

2.3 Exploratory Objective(s)

- To evaluate whether baseline values or subsequent changes in circulating immunologic parameters (including but not limited to the number of T, B and NK cells; the number of T cell subsets; soluble circulating cytokines) are associated with outcome;
- To assess neurologic functioning in the treatment arms using the Neurologic Assessment in Neuro-Oncology (NANO)

- To assess the perfusion and diffusion base imaging to correlate with changes and response to nivolumab when administered with standard and reduced bevacizumab dosing
- To assess response using the immunotherapy response assessment in neuro-oncology criteria relative to survival.

3.0 Investigational Plan

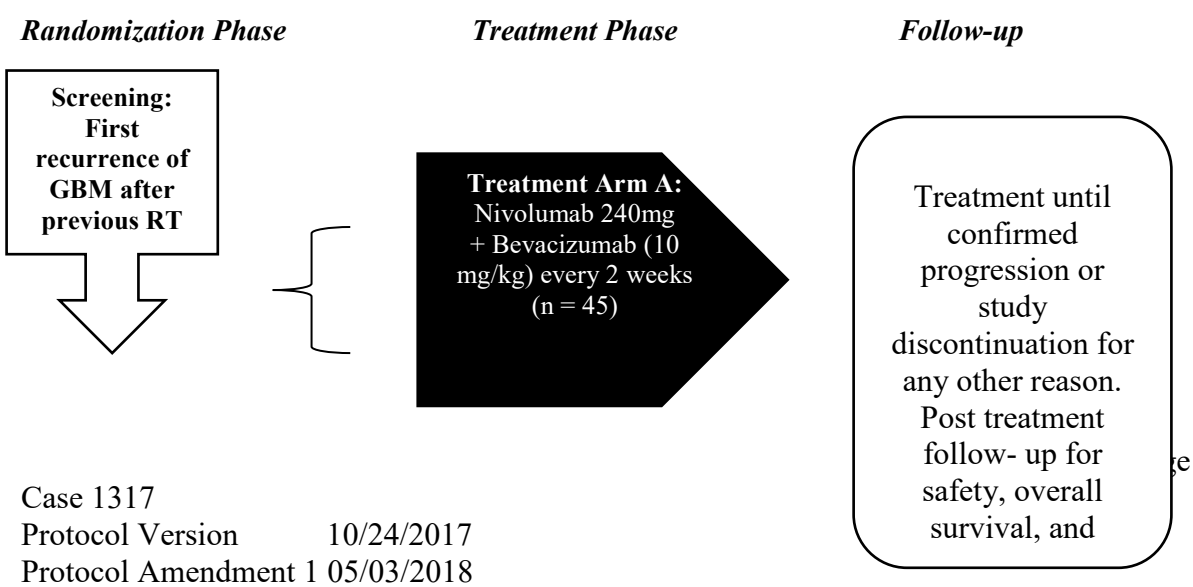
3.1 Study design and duration

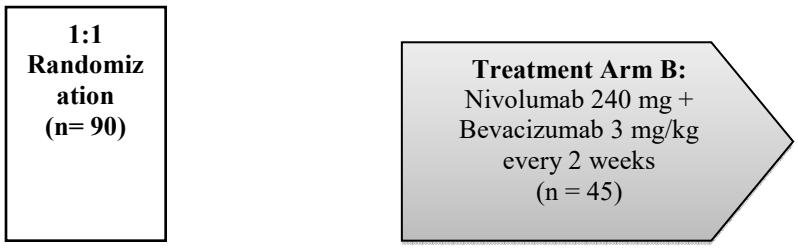
This is a randomized, open-label, phase 2 safety study of nivolumab and bevacizumab administered according to standard and reduced dosage schedules in adult (≥ 18 years) subjects with a first recurrence of glioblastoma (GBM). Subjects must have received previous treatment with radiotherapy and may have up to 2 recurrences. Subjects will undergo 1:1 randomization to receive treatment with either nivolumab (240 mg flat dosing IV every 2 weeks) and bevacizumab administered according to standard (10 mg/kg IV every 2 weeks; Arm A) and reduced (3 mg/kg IV every 2 weeks; Arm B) dosage schedules for recurrent glioblastoma subjects. The study will allow subjects that require decadron up to 4 mg/ day to participate in the study.

All subjects will be followed for safety and tolerability, tumor progression and overall survival. Tumor progression or response endpoints will be assessed using the Radiologic Assessment in Neuro-Oncology criteria and an exploratory endpoint will evaluate the response endpoints using the Immunotherapy Radiologic Assessment in Neuro-Oncology criteria (iRANO)[65] as described (Refer to Appendix 2). Treatment with study medication will continue until confirmed tumor progression, unacceptable toxicity, death, or other discontinuation criteria as specific in section 7.7, whichever comes first. A Data Safety Monitoring Committee (DMC) will meet regularly during the study to ensure that subject safety is carefully monitored.

It is expected that enrollment and follow-up of randomized subjects (45 subjects in each arm) will take approximately 12months. The study design schematic is presented in Figure 4.1-1

Figure 4.1-1: Study Design Schematic





3.2 Number of Subjects

This study consists of two arms to evaluate the anti-tumor activity of nivolumab and bevacizumab administered according to standard and low dosage schedules in subjects with recurrent glioblastoma (in first recurrence). For this purpose, approximately 90 subjects will be randomized at 1:1 ratio to arm A (nivolumab plus standard bevacizumab) or arm B (nivolumab plus low dose bevacizumab).

Arm A (nivolumab plus standard bevacizumab)
This arm will enroll 45 subjects in arm A. These subjects will receive nivolumab 240 mg and bevacizumab 10 mg/kg every 2 weeks until disease progression or unacceptable toxicity.

Arm B (nivolumab + low dose bevacizumab)
This arm will enroll 45 subjects in arm B. These subjects will receive nivolumab 240 mg and bevacizumab 3mg/kg every 2 weeks until disease progression or unacceptable toxicity.

3.3 Study Phases

This study will consist of 3 phases: screening, treatment, and follow-up. After confirmed progression or study discontinuation for any other reason, study treatment will be discontinued and subjects with enter the post-treatment follow-up phase to assess safety, progression, and overall survival.

Screening Phase:

- Begins by establishing the subject's initial eligibility and signing of the informed consent form (ICF).
- Subject is enrolled using the OnCore™ Database.

Treatment Phase:

- The patient will be randomized and assigned to one of the two arms of treatment.
- Within 3 working days from treatment assignment, the subject must receive the first dose of study medication:
    - Arm A (nivolumab + standard bevacizumab): Nivolumab 240 mg IV plus bevacizumab 10 mg/kg every two weeks.
    - Arm B (nivolumab + low dose bevacizumab): Nivolumab 240 mg IV combined with bevacizumab 3mg/kg IV every two weeks.

    For both treatment arms, nivolumab is to be administered first.
    The second infusion will be bevacizumab, and will start no sooner than 10 minutes after completion of the nivolumab infusion.

- Adverse event assessments will be documented at each visit throughout the study.
- All of the laboratory tests and vital signs will be collected prior to study drug dosing at the time points specified in Section 9.2.
- Study drug dosing may be delayed for toxicity. See Section 6.6.
- Treated subjects will be evaluated for response by the investigator and according to the iRANO criteria.[65] Tumor assessments will be performed every 8 weeks $\pm$ 1 week) until disease progression or treatment discontinuation, whichever occurs later.
- Treated subjects will be evaluated for neurologic functioning by the investigator and according to the NANO scale. Assessments will be performed every 8 weeks ($\pm$ 1 week) until disease progression or treatment discontinuation, whichever occurs later.

This phase ends when the subject experiences a confirmed tumor progression, unacceptable toxicity, or other discontinuation criteria, whichever occurs first.

Follow-Up Phase:

- Begins when the decision to discontinue a subject from study therapy is made (no further treatment with study therapy).

- Subjects who discontinue treatment for reasons other than tumor progression will continue to have tumor assessments every 8 (± 1 week) weeks until disease progression or, withdrawal of consent. All radiologically determined disease progression must be confirmed by an additional confirmatory MRI scan approximately 12 weeks following the initial assessment of radiological progression. Investigators may obtain additional follow-up MRI scans prior to 12 weeks as medically appropriate.
- Subjects will be followed for drug-related toxicities until these toxicities resolve, return to baseline or are deemed irreversible. All adverse events will be documented for a minimum of 100 days after last dose.
- After completion of the first two follow-up visits, subjects will be followed every 3 months for survival.

3.4 Method of Assigning Subject Identification

This protocol is a randomized study. After verifying each patient's eligibility status and administering informed consent, the patient will be enrolled into the study by the study coordinator or research nurse to obtain the subject number. Every subject that signs the informed consent form must be assigned a subject number. The investigator or designee will register the subject for enrollment by following the enrollment procedures established by the principal investigator. If the subject withdraws or screen fails before starting treatment, the assigned subject number will not be re-issued. The following information is required for enrollment:

- Date that informed consent was obtained
- Date of birth
- Gender at birth

After enrollment, Enrolled subjects who have met all eligibility criteria will be ready to be randomized by the study coordinator. The following information is required for subject randomization:

- Subject number
- Date of birth
- Methylation status
- Surgery type (complete resection, near complete resection, or biopsy)
- KPS- Karnofsky Performance Scale

Subjects meeting all eligibility criteria will be randomized in a 1:1 ratio to either treatment arm A (nivolumab + standard bevacizumab) or arm B (nivolumab + low dose bevacizumab).

3.5 Review of Safety

The subjects' safety will be monitored on an ongoing basis. Safety meeting will be done every 4-8 weeks depending on patient accrual. An independent Data Monitoring Committee (DMC) will provide safety reviews every six months. Decisions regarding safety will be made by the sponsor (Cleveland Clinic) in discussions with site investigators and BMS. In addition, a BMS medical safety team (MST) will routinely reviews safety signals across the entire nivolumab program and inform the team at Cleveland Clinic. The DMC will review all available data (safety and efficacy) and will recommend continuation, modification or termination of the study protocol based upon their review.

4.0 Patient Selection

Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. The checklist must be completed for each patient and must be signed and dated by the treating physician.

Patient's Initials _____

Patient ID_____

Research Nurse /
Study Coordinator Signature: _____ **Date** _____

Treating Physician [Print] _____

Treating Physician Signature: _____ **Date** _____

4.1 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment:

1. Signed Written Informed Consent

a) Written informed consent and HIPAA authorization obtained from the subject/legal representative prior to
performing any protocol-related procedures, including screening evaluations

b) Subjects must be willing and able to comply with scheduled visits, treatment schedule, laboratory testing, and other requirements of the study, including disease assessment by MRI.

2. Target Population

a) Histologically confirmed diagnosis of supratentorial glioblastoma
b) Age ≥ 18 years old
c) Previous first line treatment with at least radiotherapy
d) Documented first recurrence of GBM by diagnostic biopsy or contrast enhanced magnetic resonance imaging (MRI) performed within 21 days of randomization per RANO criteria.
e) If first recurrence of GBM is documented by MRI, an interval of at least 12 weeks after the end of prior radiation therapy is required unless there is either:
    a. histopathologic confirmation of recurrent tumor, or
    b. new enhancement on MRI outside of the radiotherapy treatment field
f) An interval of ≥ 28 days and full recovery (i.e., no ongoing safety issues) from surgical resection prior to randomization.
g) Karnofsky performance status of 70 or higher (Appendix 1)
h) Life expectancy ≥ 12 weeks
i) Up to ten unstained slides of 5 microns thickness or a block of tissue will be required to be sent if tissue is available. If the tissue is not available then Principal investigator permissions is required prior to enrollment.

3. Age and Reproductive Status

a) Men and women, age ≥ 18 years old at the time of screening
b) Women of childbearing potential (WOCBP, as defined in Section 5.4) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 1 day prior to the start of study drug

c) Women must not be breastfeeding
d) WOCBP must use appropriate method(s) of contraception from the time of enrollment for the duration of treatment with study drug (s) plus 5 half-lives of study drug (s) plus 6 months post treatment completion for a treatment arm A (nivolumab + standard dose bevacizumab)and treatment arm B (nivolumab + low dose bevacizumab).
e) Men who are sexually active with WOCBP must use contraceptive method such as male condom with spermicide. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for the duration of treatment with study drug (s) plus 5 half-lives of study drug (s) plus 90 days (duration of sperm turnover) for a total of 31 weeks post-treatment completion.
f) Women who are not of childbearing potential (i.e., who are postmenopausal or surgically sterile as well as azoospermic men) do not require contraception.
g) Azoospermic males and WOCBP who are continuously not heterosexually active are exempt from contraceptive requirements.

Investigators shall counsel WOCBP and male subjects who are sexually active with WOCBP on the importance of pregnancy prevention and the implications of an unexpected pregnancy. Investigators shall advise WOCBP and male subjects who are sexually active with WOCBP on the use of highly effective methods of contraception. Highly effective methods of contraception have a failure rate of < 1% per year when used consistently and correctly.

At a minimum, subjects must agree to the use of two methods of contraception, with one method being highly effective and the other method being either highly effective or less effective as listed below:

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

- Male condoms with spermicide
- Hormonal methods of contraception including combined oral contraceptive pills, vaginal ring, injectables, implants, and intrauterine devices (IUDs) such as Mirena® by WOCBP subjects or male subject's WOCBP partner.
- Nonhormonal IUDs, such as ParaGard
- Tubal ligation
- Vasectomy.
- Complete Abstinence*

*Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Subjects who choose complete abstinence

are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence.

LESS EFFECTIVE METHODS OF CONTRACEPTION

- Diaphragm with spermicide
- Cervical cap with spermicide
- Vaginal sponge
- Male Condom without spermicide*
- Progestin only pills by WOCBP subjects or male subject's WOCBP partner
- Female Condom*

*A male and female condom must not be used together

Women of Child Bearing Potential (WOCBP)

A women of childbearing potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal. Menopause is defined as 12 months of amenorrhea in a woman over age 45 years in the absence of other biological or physiological causes. In addition, women under the age of 55 years must have a serum follicle stimulating hormone, (FSH) level > 40mIU/mL to confirm menopause.*

*Women treated with hormone replacement therapy, (HRT) are likely to have artificially suppressed FSH levels and may require a washout period in order to obtain a physiologic FSH level. The duration of the washout period is a function of the type of HRT used. The duration of the washout period below are suggested guidelines and the investigators should use their judgment in checking serum FSH levels. If the serum FSH level is > 40 mIU/ml at any time during the washout period, the woman can be considered postmenopausal:

- 1 week minimum for vaginal hormonal products (rings, creams, gels)
- 4 week minimum for transdermal products
- 8 week minimum for oral products

Other parenteral products may require washout periods as long as 6 months
Each of the criteria in the checklist that follows must be met in order for a patient to be considered eligible for this study. Use the checklist to confirm a patient's eligibility. The

checklist must be completed for each patient and must be signed and dated by the treating physician.

Recovery from the toxic effects of prior therapy, with a minimum time of:

- ≥ 28 days elapsed from the administration of any investigational agent
- ≥ 28 days elapsed from the administration of any prior cytotoxic agents, except
- ≥ 14 days from vincristine, ≥ 21 days from procarbazine, and ≥ 42 days from
- nitrosureas
- ≥ 14 days elapsed from administration of any non-cytotoxic agent (e.g., interferon, tamoxifen, thalidomide, cis-retinoic acid)

4. Physical and Laboratory Test Findings

Screening/Baseline laboratory values must meet the following criteria (using CTCAE v5.0):

- WBC ≥ 2000/uL
- Neutrophils ≥ 1500/uL
- Platelets ≥ $100x10^3$/uL
- Hemoglobin ≥ 9.0 g/dL
- Serum creatinine ≤ 1.5 x ULN or creatinine clearance (CrCl) ≥ 40 mL/min (using the Cockcroft-Gault formula)
  Female CrCl = (140 - age in years) x weight in kg x 0.85 /72 x serum creatinine in mg/dL
  Male CrCl = (140 - age in years) x weight in kg x 1.00/72 x serum creatinine in mg/dL
- AST ≤ 3x ULN
- ALT≤ 3x ULN
- Pregnancy test (serum)
- Bilirubin ≤ 1.5x ULN (except subjects with Gilbert Syndrome, who can have
  - total bilirubin < 3.0 mg/dL)

4.2 Exclusion Criteria

Subjects with any of the following are ineligible for this research study:

1. Target Disease Exceptions

a. More than one recurrence of GBM

b. Presence of extracranial metastatic, significant leptomeningeal disease or tumors primarily localized to the brainstem or spinal cord.

2. Medical History and Concurrent Diseases

a. Any serious or uncontrolled medical disorder that, in the opinion of the investigator, may increase the risk associated with study participation or study drug administration, impair the ability of the subject to receive protocol therapy, or interfere with the interpretation of study results.
b. Subjects with active, known or suspected autoimmune disease. Subjects with vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring chronic and systemic immunosuppressive treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll. Subjects have any other condition requiring systemic treatment with corticosteroids or other immunosuppressive agents within 14 days. Inhaled or topical steroids and adrenal replacement doses >10mg daily prednisone equivalent are permitted in absence of active autoimmune disease.
c. Previous radiation therapy with anything other than standard radiation therapy (i.e., focally directed radiation) administered as first line therapy.
d. Previous treatment with carmustine wafer except when administered as first line treatment and at least 6 months prior to randomization
e. Previous bevacizumab or other VEGF or anti-angiogenic treatment
f. Previous treatment with a PD-1, PD-L1 or CTLA-4 targeted therapy
g. Evidence of > Grade 1 CNS hemorrhage on the baseline MRI scan
h. Inadequately controlled hypertension (defined as systolic blood pressure ≥160 mmHg and /or diastolic blood pressure ≥100 mmHg) within 7 days of first study treatment
i. Prior history of hypertensive crisis, hypertensive encephalopathy, reversible posterior leukoencephalopathy syndrome (RPLS);
j. Prior history of gastrointestinal diverticulitis, perforation, or abscess;
k. Clinically significant (i.e., active) cardiovascular disease, for example cerebrovascular accidents ≤ 6 months prior to study enrollment, myocardial infarction ≤ 6 months prior to study enrollment, unstable angina, New York Heart Association (NYHA) Grade II or greater congestive heart failure (CHF), or serious cardiac arrhythmia uncontrolled by medication or potentially interfering with protocol treatment;
l. Significant vascular disease (e.g., aortic aneurysm requiring surgical repair or recent arterial thrombosis) within 6 months prior to start of study treatment. Any previous venous thromboembolism ≥ NCI CTCAE Grade 3 within 3 months prior to start of study treatment;

m. History of pulmonary hemorrhage/hemoptysis ≥ grade 2 (defined as ≥ 2.5 mL bright red blood per episode) within 1 month prior to randomization;
n. History or evidence of inherited bleeding diathesis or significant coagulopathy at risk of bleeding (i.e., in the absence of therapeutic anticoagulation);
o. Current or recent (within 10 days of study enrollment) use of anticoagulants that, in the opinion of the investigator, would place the subject at significant risk for bleeding. Prophylactic use of anticoagulants is allowed;
p. Surgical procedure (including open biopsy, surgical resection, wound revision, or any other major surgery involving entry into a body cavity) or significant traumatic injury within 28 days prior to first study treatment, or anticipation of need for major surgical procedure during the course of the study;
q. Minor surgical procedure (e.g., stereotactic biopsy within 7 days of first study treatment; placement of a vascular access device within 2 days of first study treatment);
r. History of intracranial abscess within 6 months prior to randomization;
s. History of active gastrointestinal bleeding within 6 months prior to randomization;
t. Serious, non-healing wound, active ulcer, or untreated bone fracture;
u. Subjects unable (due to existent medical condition, e.g., pacemaker or ICD device) or unwilling to have a head contrast enhanced MRI

3. Physical and Laboratory Test Findings

a. Positive test for hepatitis B virus surface antigen (HBV sAg) or detectable hepatitis C virus ribonucleic acid (HCV RNA) indicating acute or chronic infection
b. Known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS)

4. Allergies and Adverse Drug Reaction

c. History of severe hypersensitivity reaction to any monoclonal antibody

5. Corticosteroid Use

d. Subjects that require decadron > 4 mg/ day or equivalent of steroids

4.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.4 Women of Child Bearing Potential (WOCBP)

A women of childbearing potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal. Menopause is defined as 12 months of amenorrhea in a woman over age 45 years in the absence of other biological or physiological causes. In addition, women under the age of 55 years must have a serum follicle stimulating hormone, (FSH) level > 40mIU/mL to confirm menopause.*

*Women treated with hormone replacement therapy, (HRT) are likely to have artificially suppressed FSH levels and may require a washout period in order to obtain a physiologic FSH level. The duration of the washout period is a function of the type of HRT used. The duration of the washout period below are suggested guidelines and the investigators should use their judgment in checking serum FSH levels. If the serum FSH level is > 40 mIU/ml at any time during the washout period, the woman can be considered postmenopausal:

- 1 week minimum for vaginal hormonal products (rings, creams, gels)
- 4 week minimum for transdermal products
- 8 week minimum for oral products

4.5 Concomitant Treatments

4.5.1 Steroids

This study allows subjects to receive systemic corticosteroid therapy consisting of dexamethasone up to 4 mg/day (or other dexamethasone-equivalent therapy) at entry.

For subjects receiving study therapy, systemic corticosteroid at a dose higher than 4 mg/day or physiologic replacement doses of steroids are permitted for:

a) treatment-related AEs;
b) sequelae of underlying GBM treatment; or
c) treatment of non-autoimmune conditions (such as prophylaxis for contrast dye allergy, delayed-type hypersensitivity reaction caused by contact allergen).

Details regarding corticosteroid use prior to and during the study will be collected (name of medication, doses utilized, start and stop dates, frequency of use, route of administration). Information regarding concomitant corticosteroid use may be analyzed with regard to study outcome measures. Subjects should be maintained on as low a dose of corticosteroids administered for as short a time period as possible. If medically appropriate, subjects should be tapered off corticosteroids whenever possible.

Subjects requiring chronic treatment with corticosteroids should be treated with histamine-2-receptor antagonists or proton pump inhibitors as prophylaxis for potential gastrointestinal adverse reactions (ulceration, perforation, hemorrhage) unless otherwise contraindicated.

4.5.2 Other Permitted Therapy

Other concomitant medications such as anti-seizures medications and supportive care measures for treating depression, anxiety, and fatigue are permitted as the discretion of the treating physician.

As there is potential for hepatic toxicity with nivolumab, drugs with a predisposition to hepatotoxicity should be used with caution in all study subjects (Appendix 6 – add the attached list as Appendix 6 before the Algorithms.

Concomitant medications are recorded at baseline and throughout the treatment phase of the study in the appropriate section of the CRF. All medications (prescriptions or over the counter medications) continued at the start of the study or started during the study and different from the study drug must be documented in the concomitant therapy section of the CRF.

4.5.3 Prohibited and/or Restricted Treatments

The following medications are prohibited during the study:

- Any concurrent drug or other investigational agents for treatment of GBM (ie, chemotherapy, hormonal therapy, immunotherapy, radiation therapy)
- Medications contraindicated with bevacizumab treatment (refer to the package insert, summary of product characteristics (SmPC), or similar document)
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.

Supportive care for disease-related symptoms may be offered to all subjects on the study.

4.5.4 Other Restrictions and Precautions

Study related MRI imaging of the brain will be performed based on schedule as defined in this protocol. Investigators may obtain additional follow-up MRI scans as medically indicated. It is

the local imaging facility's responsibility to determine, based on subject attributes (e.g., allergy history, diabetic history and renal status), the appropriate imaging modality and contrast regimen for each subject. Subjects with renal insufficiency should be assessed as to whether or not they should receive contrast and if so, what type and dose of contrast is appropriate. Specific to MRI, subjects with severe renal insufficiency (ie, estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73m2) are at increased risk of nephrogenic systemic fibrosis. MRI contrast should not be given to this subject population.

5.0 Registration

All subjects who have been consented are to be registered in the OnCore™ Database. Randomization will be completed per Taussig standard procedure. For those subjects who are consented, but not enrolled, the reason for exclusion must be recorded.

All subjects will be registered through Cleveland Clinic and will be provided a study number by contacting the study coordinator listed on the cover page.

The Advarra EDC™ and OnCore™ databases will be utilized, as required by the Case Comprehensive Cancer Center and Cleveland Clinic, to provide data collection for both accrual entry and trial data management. Advarra EDC and OnCore™ are Clinical Trials Management Systems housed on secure servers. Access to data through Advarra EDC and OnCore™ is restricted by user accounts and assigned roles. Once logged into the Advarra EDC or OnCore™ system with a user ID and password, Advarra EDC™ and OnCore™ define roles for each user which limits access to appropriate data. Applications for user accounts can be obtained by contacting the OnCore™ Administrator at OnCore-registration@case.edu for OnCore™ access, and taussigoncore@ccf.org for Advarra EDC™ access.

Advarra EDC™ is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. When properly utilized, Advarra EDC™ is 21 CFR 11 compliant. This study will utilize electronic Case Report Form completion in the Advarra EDC™ database. A calendar of events and required forms are available in Advarra EDC™.

6.0 Treatment

Study drugs include both Non-investigational (NIMP) and Investigational Medicinal Products (IMP) and can consist of the following:

- All products, active or placebo, being tested or used as a comparator in a clinical trial.
- Study required pre-medication, and

- Other drugs administered as part of the study that are critical to claims of efficacy
- (e.g., background therapy, rescue medications)
- Diagnostic agents: (such as glucose for glucose challenge) given as part of the protocol requirements must also be included in the dosing data collection

6.1 Investigational Products

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

The investigational product should be stored in a secure area according to local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations.

In this protocol, the investigational products are BMS-936558 (nivolumab) and bevacizumab. Investigational product information is provided in Table 7.1-1.
BMS is supplying the study drug BMS-936558 (nivolumab). Bevacizumab may be obtained by the investigational sites located in the USA as a local commercial product (which may be available as a different potency/package size than listed in Table 7.1-1) if local regulations allow and agreed to by BMS.

Table 7.1-1 Investigational Product Description

Product Description and Dosage Form	Potency	Primary Packaging (Volume)/Label Type	Secondary Packaging (Qty)/Label Type	Appearance	Storage Conditions (per label)
Nivolumab* (BMS-936558-01): Injection drug product is a sterile, non-pyrogenic, single-use,	100 mg/vial (10 mg/mL)	10-mL Type 1 flint glass vials stoppered with butyl stoppers and sealed with aluminum seals / Open-label	5 or 10 vials per carton / Open-label	Clear to opalescent colorless to pale yellow liquid. May contain	2 to 8°C (36 to 46°F). Protect from light and freezing

isotonic aqueous solution**				particles	
Bevacizumab: Solution for infusion	400 mg/vial	16mL per vial / Open-label	1 vial per carton / Open-label	Clear to slightly opalescent, colorless to pale brown liquid	2 to 8°C (36 to 46°F). Protect from light and freezing. Do not shake.

* Note other names = MDX-1106, ONO-4538, anti-PD-1
**Nivolumab may be labeled as BMS-936558-01 Solution for Injection

6.2 Non-investigational Products

Other medications used as support or escape medication for preventative, diagnostic, or therapeutic reasons, as components of the standard of care for a given diagnosis, may be considered as non-investigational products.

In this protocol, non-investigational product(s) is/are: Not applicable for this study.

6.3 Storage, Handling and Dispensing of Investigational Products

The investigator should ensure that the study drug is stored in accordance with the environmental conditions (temperature, light, and humidity) as per product information and the Investigator Brochure and per local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations. If concerns regarding the quality or appearance of the study drug arise, the study drug should not be dispensed and contact BMS immediately.

Investigational product documentation will be maintained that includes all processes required to ensure drug is accurately administered. This includes documentation of drug storage, administration and, as applicable, storage temperatures, reconstitution, and use of required processes (e.g., required diluents, administration sets).

Infusion-related supplies (e.g., IV bags, in-line filters, 0.9% NaCl solution) will not be supplied by the sponsor and should be purchased locally if permitted by local regulations.

For non-investigational product, if marketed product is utilized, it should be stored in accordance with the package insert, summary of product characteristics (SmPC), or similar.

Please refer to the current version of the Investigator Brochure and/or shipment reference sheets for additional information on storage, handling, dispensing, and infusion information for nivolumab.

6.3.1 Nivolumab (BMS-936558)

Nivolumab is an injection drug product. This product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution formulated at 10 mg/mL (100mg/vial). Vials should be stored at 2 to 8 degrees C and should be protected from light and freezing. If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.

For additional details on prepared drug storage and use time of nivolumab under room temperature/light and refrigeration, please refer to the BMS-936558 (nivolumab) Investigator Brochure section for "Recommended Storage and Use Conditions"

6.3.2 Bevacizumab

Please refer to the package insert, summary of product characteristics (SmPC), or similar document for details regarding drug preparation, administration, and use time.

6.4 Destruction or Return of Investigational Products

For this study, the investigational products study drugs such as partially used study drug containers, vials and syringes may be destroyed on site.

Any unused study drugs can only be destroyed after being inspected and to be reconciled by the site, and destroyed according to institution SOP. Drug accountability and destruction logs to be sent to BMS

On-site destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and
- regulations, including any special requirements for controlled or hazardous substances.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the

containers. The method of disposal, i.e., incinerator, licensed sanitary landfill, or licensed waste disposal vendor must be documented.

- Accountability and disposal records are complete, up-to-date, and available for the Monitor to review throughout the clinical trial period.

6.5 Study Drug Administration

6.5.1 Dosing Schedule and Administration

The dosing regimen and schedule for Arm A (nivolumab plus standard bevacizumab) and Arm B (nivolumab plus low dose bevacizumab) are detailed in Tables 6.5-1 and 6.5-2.

Table 6.5.1-1: Dosing Schedule for Arm A: Nivolumab (BMS-936558) plus standard bevacizumab	
Every 2 week dosing	
Day 1, Week 1	**Day 1, Week 3 and every two weeks thereafter**
240 mg IV nivolumab + 10 mg/kg IV bevacizumab	240 mg IV nivolumab + 10 mg/kg IV bevacizumab

Table 6.5.1-2: Dosing Schedule for Arm B: Nivolumab (BMS-936558) plus low dose Bevacizumab	
Every 2 week dosing	
Day 1, Week 1	**Day 1, Week 3 and every two weeks thereafter**
240 mg IV nivolumab + 3 mg/kg IV bevacizumab	240 mg IV nivolumab + 3 mg/kg IV bevacizumab

Nivolumab will be given every two weeks at a dose of 240 mg. Nivolumab will be administered as a 30-minute IV infusion, using a volumetric pump with a 0.2/0.22 micron in-line filter at the protocol-specified dose. The drug can be diluted with 0.9% normal saline for delivery but the total drug concentration cannot be below 0.35 mg/ml. The drug is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

Bevacizumab will be given every two weeks with nivolumab as an IV infusion at a dose of 10mg/kg for subjects in Arm A and at 3 mg/kg for subjects in Arm B. Nivolumab and bevacizumab will be prepared separately and administered in separate infusion bags including appropriate filtering. Nivolumab is to be administered first. The second infusion will be bevacizumab, and will start no sooner than 10 minutes after completion of the nivolumab infusion.

6.5.2 Dosing Calculation

Each dose of nivolumab and bevacizumab will be administered every 2 weeks (+/- 3 days), but subjects may be dosed no less than 12 days from the previous dose of drug. Nivolumab will be dosed at a flat dose of 240 mg every 2 weeks. Bevacizumab will be dosed at 10 mg/kg for subjects on Arm A and at 3 mg/kg for subjects on Arm B. The dose of bevacizumab will be based on weight at screening and will be recalculated if there is a >10% change in body weight during the study or per institutional guidelines.

6.5.3 Dosing Modifications

Dosing modifications, including dose reductions or dose escalations, are not permitted.

Dosing delay is allowed for toxicity management, as specified in section 6.6.

6.5.4 Antiemetic Pre-medications

Antiemetic pre-medications should not be routinely administered prior to dosing of drugs. See Section 6.8 for premedication recommendations following a nivolumab related infusion reaction.

6.6 Dose Delay Criteria

6.6.1 Dose Delay Criteria for Nivolumab

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab). All study drugs must be delayed until treatment can resume.

Nivolumab administration should be delayed for the following:

Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:

- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related AE
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:
    - Grade 3 lymphopenia or leukopenia does not require dose delay.

- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity.
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity.

- Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

6.6.2 Criteria to Delay or Discontinue Bevacizumab

Guidance for when bevacizumab administration should be delayed or discontinued should be in accordance with the package insert or summary of product characteristics (SmPC).

There are no reductions in the bevacizumab dose. Specific guidelines for bevacizumab dose management due to adverse events considered at least possibly related to bevacizumab are summarized in Table 8. If adverse events occur that require holding bevacizumab, the dose will remain the same once treatment resumes. Any toxicity associated or possibly associated with bevacizumab treatment should be managed according to standard medical practice, unless listed in Table 8 below. Bevacizumab has a terminal half-life of 2 to 3 weeks; therefore, its discontinuation results in slow elimination over several months. There is no available antidote for bevacizumab.

Subjects should be assessed clinically for toxicity prior to, during, and after each infusion. If unmanageable toxicity occurs because of bevacizumab at any time during the study, treatment with bevacizumab should be discontinued.

Adverse events requiring delays or permanent discontinuation of bevacizumab are listed in Table 8.

Table 8 Bevacizumab Dose Management Due to Adverse Events

Event	Action to be Taken
Hypertension No dose modifications for grade 1/2 events	
Grade 3	If not controlled to ≤ 159/99 mmHg with medication, discontinue bevacizumab.

Grade 4 (including RPLS (confirmed by MRI) or hypertensive encephalopathy)	Discontinue bevacizumab.
Hemorrhage No dose modifications for grade 1 non-CNS events	
Grade ≥ 1 New CNS hemorrhage	Discontinue bevacizumab.
Grade > 1 non-CNS hemorrhage	Discontinue bevacizumab.
Venous Thrombosis No dose modifications for grade 1/2 events	
Grade 3/ Asymptomatic Grade 4	Hold study drug treatment. If the planned duration of full-dose anticoagulation is <2 weeks, study drug should be held until the full-dose anticoagulation period is over. If the planned duration of full-dose anticoagulation is ≥2 weeks, study drug may be resumed during the period of full-dose anticoagulation if the following criteria is met: • The participant must be therapeutically anti-coagulated with an approved anticoagulant agent according to standard prescribing guidelines.
Symptomatic Grade 4	Discontinue bevacizumab.
Arterial Thromboembolic event (Angina, myocardial infarction, transient ischemic attack, cerebrovascular accident, and any other arterial thromboembolic event)	
Any grade	Discontinue bevacizumab.
Congestive Heart Failure (Left ventricular systolic dysfunction) No dose modifications for grade 1/2 events	
Grade 3 Grade 4	Hold bevacizumab until resolution to Grade ≤ 1. Discontinue bevacizumab.
Proteinuria No dose modifications for grade 1/2 events	
Grade 3	Hold bevacizumab treatment until ≤ Grade 2, as determined by 24 hr collection ≤ 3.5 g

Grade 4 (nephrotic syndrome)	Discontinue bevacizumab
GI Perforation	Discontinue bevacizumab.
Bowel Obstruction	
Grade 1	Continue patient on study for partial obstruction NOT requiring medical intervention.
Grade 2	Hold bevacizumab for partial obstruction requiring medical intervention. Patient may restart upon complete resolution.
Grade 3/4	Hold bevacizumab for complete obstruction. If surgery is necessary, patient may restart bevacizumab after full recovery from surgery, and at investigator's discretion.
Wound dehiscence requiring medical or surgical therapy	Discontinue bevacizumab.
Infusion Related Reaction	
Grade 1/2	Slow infusion to 50% or less or interrupt. When symptoms have completely resolved, the infusion may be continued at not more than 50% of the rate prior to the reaction and increased in 50% increments every 30 minutes if well tolerated. Infusions may be restarted at the full rate during the next cycle.
Grade 3/4	Discontinue bevacizumab.
Other Unspecified Bevacizumab-Related Adverse Events	
Grade 3	Hold bevacizumab until recovery to ≤ Grade 1
Grade 4	Discontinue bevacizumab.

6.6.3 Criteria to Resume Treatment

Subjects may resume treatment with bevacizumab as summarized in Table 8. Subjects may resume treatment with nivolumab when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity

- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the next scheduled time point will be delayed until dosing resumes.

If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section 6.7.

6.6.4 Management Algorithms

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

- Gastrointestinal
- Renal
- Pulmonary
- Hepatic
- Endocrinopathies
- Skin
- Neurological.

While the nivolumab investigator brochure contains safety management algorithms for similar adverse events, the recommendations are to follow the nivolumab algorithms for immune-oncology agents (I-O) in order to standardize the safety management. Therefore, the algorithms recommended for utilization in this protocol are included in Appendix 6. The guidance provided in these algorithms should not replace the Investigator's medical judgment but should complement it.

6.7 Discontinuation Criteria

Subjects who require nivolumab discontinuation due to toxicity are permitted to continue to receive study treatment with bevacizumab alone. Conversely, subjects who require bevacizumab discontinuation due to toxicity are permitted to continue to receive study therapy with nivolumab alone. Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
            - AST or ALT > 8 x ULN
            - Total bilirubin > 5 x ULN
            - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN

- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
    - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequellae and are corrected with supplementation/appropriate management within 72 hours of their onset
- Any dosing interruption lasting > 6 weeks with the following exceptions:
    - Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
    - Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing

6.8 Treatment of Nivolumab Related Infusion Reactions

Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE v5.0 guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated)

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.

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

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

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

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected

slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

6.9 Cerebral Edema

Due to the immunologic nature of nivolumab, cerebral edema could theoretically result as a consequence of nivolumab administration due to immune infiltration of the brain. Symptoms related to cerebral edema may include headache or neurologic deficit that is either new or worsened. Subjects with any signs or symptoms of cerebral edema should be treated as clinically appropriate including initiation or increased systemic corticosteroid dosing, treatment with an osmotic diuretic or surgical decompression. Subsequent nivolumab dosing should be immediately interrupted if significant clinical symptoms attributable to cerebral edema develop. Treatment with additional nivolumab doses may only be re-initiated if clinically significant symptoms attributable to cerebral edema have resolved to grade $\leq$ 1 or pre-treatment baseline. Subjects who develop CTCAE v5.0 grade 4 cerebral edema attributable to nivolumab administration should not receive further nivolumab doses but may continue study therapy bevacizumab.

6.10 Treatment Beyond Initial Radiologic Assessment of Disease Progression

Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of PD. Similarly, in the GBM patient population, it is well-known that a subset of subjects who receive standard of care including upfront radiation therapy and temozolomide will go on to demonstrate "pseudo-progression". This phenomenon describes the transient increase in tumor enhancement on contrast MRI, which eventually returns to baseline without any change in therapy, unlike true tumor progression.

As it can be challenging to distinguish disease progression from pseudo-progression, and to avoid premature discontinuation of study drug, subjects with radiographic evidence of progressive disease within six months of initiating study therapy and who are not experiencing significant neurologic decline, should remain on study pending radiographic confirmation of tumor progression on follow-up imaging obtained three months later as specified in the iRANO criteria.[65] If radiographic confirmation is obtained on follow-up imaging, the date of tumor progression will be back-dated to the date of the scan that initially demonstrated progression. Thus, this protocol will allow for continuation of the study drug beyond initial demonstration of tumor progression pending confirmation of progression.

Subjects will be permitted to continue treatment beyond initial iRANO-defined PD as long as they meet the following criteria:

- Investigator-assessed clinical benefit and
- Subject is tolerating study drug and is not demonstrating significant neurologic decline felt to be attributable to underlying tumor growth.

Subjects with confirmed progression on follow-up imaging three months later or who develop significant neurologic decline felt to be attributable to underlying tumor growth, 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 subject met the criteria for progression.

If radiologic progression cannot be differentiated from pseudoprogression, the investigator may recommend that patient undergo a surgical resection to assess histopathology. In this case, tumor biopsy samples (blocks 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 subject will be discontinued from study medication per protocol discontinuation criteria, and the date of progression will be the day that it was first suspected. If tumor pathology reveals treatment-related changes and does not confirm disease progression, the subject may continue study medication. An MRI after the resection is required prior to treatment continuation. The subject will then continue all on-treatment tumor assessments as per the treatment schedule described in this protocol.

6.10.1 Central Neuropathologic Review of Tumor Samples After Biopsy or Resection

Representative tumor tissue samples will be reviewed locally for progression versus treatment-associated changes.

6.11 Blinding/Unblinding

Not applicable.

6.12 Treatment Compliance

In this study, treatment compliance will be monitored by drug accountability as well as the subject's medical record and eCRF

7.0 Adverse Events and Potential Risks

The following is a list of AEs (Section 7.1) and the reporting requirements associated with observed AEs (Sections 7.3 and 7.4).

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

7.1 Adverse Events and Potential Risks

7.1.1 Nivolumab

CA209003 is an ongoing Phase 1 open label, multiple dose escalation study in 306 subjects with select previously treated advanced solid tumors, including melanoma, RCC, NSCLC, colorectal cancer, and hormone-refractory prostate cancer. Subjects received nivolumab at doses of 0.1, 0.3, 1, 3, or 10 mg/kg intravenously every 2 weeks, up to a maximum of 2 years of total therapy. As of 18-Mar-2013, a total of 306 melanoma subjects were treated with nivolumab in the dose range of 0.1 - 10 mg/kg.

No maximal tolerated dose was identified in CA209003. The incidence, severity and relationship of AEs were generally similar across dose levels and tumor types. Nivolumab related AEs of any grade occurred in 75.2% of subjects. Of the 306 treated subjects, 303 (99.0%) subjects have at least 1 reported AE regardless of causality. The most frequently reported AEs were fatigue (54.9%), decreased appetite (35.0%), diarrhea (34.3%), nausea (30.1%), and cough (29.4%). Treatment-related AEs were reported in 230 (75.2%) of the 306 subjects. The most frequently reported treatment-related AEs were fatigue (28.1%), rash (14.7%), diarrhea (13.4%), and pruritus (10.5%). Most treatment-related AEs were low grade. Treatment-related high grade (Grade 3-4) AEs were reported in 52 (17.0%) of subjects. The most common treatment-related high grade AEs were fatigue (2.3%) and diarrhea (1%).

Drug-related SAEs occurred in 11.5% of subjects. Grade 3-4 drug-related SAEs reported in at least 2 subjects included: diarrhea (3 subjects, 1.0%), pneumonitis (3 subjects, 1.0%), pneumonia (2 subjects, 0.7%) and lipase increased (2 subjects, 0.7%).

Select AE categories (events with a potential inflammatory mechanism requiring more frequent monitoring and/or unique intervention such as immunosuppressants and/or endocrine replacement therapy) include: GI AEs, pulmonary AEs, renal AEs, hepatic AEs, skin AEs, and endocrinopathies. In addition, select AEs include a category for infusion reactions. Each category is composed of a discrete set of preferred terms, including those of greatest clinical relevance. These select AEs are considered events of interest based on the mechanism of action and were previously referred to as immune-related AEs or immune-mediated AEs.
The 10 mg/kg cohort had numerically greater frequency of high-grade select AEs including the subcategories of endocrinopathies, GI, pulmonary, and infusion reactions (Table 8.1.1-1). Most high grade events resolved following the treatment guidelines for the treatment of pulmonary events, GI events, hepatic events, renal events, and endocrine events, respectively.

Treatment-related AEs leading to discontinuation were reported in 32 (10.5%) of the 306 treated subjects on CA209003. The most frequent of these were pneumonitis (8 subjects; 2.6%) and colitis (3 subjects; 1.0%).There were 3 (1%) drug related deaths; each occurred after development of pneumonitis.

Additional details on the safety profile of nivolumab, including results from other clinical studies, are also available in the BMS-936558 (nivolumab) IB.
Table 8.1.1 -1 : Treatment-related Select Adverse Events by Treatment - All CTC Grades Reported in at Least 10 Treated Subjects in CA209003

Preferred Term	0.1 mg/kg n=17		0.3 mg/kg n=18		1 mg/kg n=86		3 mg/kg n=54		10 mg/kg n=131		Total N=306	
	Any Grade	Grade 3-4	Any Grade	Grade 3-4	Any Grade	Grade 3-4	Any Grade	Grade 3-4	Any Grade	Grade 3-4	Any Grade	Grade 3-4
Any Select AE	8 (47)	1 (5.9)	9 (50)	0	42 (49)	3 (4)	23 (43)	2 (4)	58 (44)	13 (10)	140 (46)	19 (6)
Any Endocrinopathies	4 (24)	0	2 (11)	0	9 (11)	0	4 (7)	0	10 (8)	3 (2)	29 (10)	3 (1)
Endocrinopathies Thyroid	3 (18)	0	2 (11)	0	9 (11)		4 (7)	0	8 (6)	2 (2)	26 (9)	2 (1)
Blood TSH increased	2 (12)	0	1 (6)	0	2 (2)	0	2 (4)	0	4 (3)	1 (1)	11 (4)	1 (0.3)
Hypothyroidism	1 (6)	0	1 (6)	0	5 (6)	0	1 (2)	0	3 (2)	1 (1)	11 (4)	1 (0.3)
Any Skin AEs	3 (18)	0	5 (28)	0	27 (31)	0	12 (22)	0	28 (21)	1 (1)	75 (25)	1 (0.3)
Rash	3 (18)	0	3 (17)	0	20 (23)	0	5 (9)	0	14 (11)	0	45 (15)	0
Pruritus	0	0	1 (6)	0	15 (17)	0	3 (6)	0	13 (10)	1 (1)	32 (11)	1 (0.3)
Any GI AE	1 (6)	0	2 (11)	0	19 (22)	0	7 (13)	0	14 (11)	3 (2)	43 (14)	3 (1)
Diarrhea	1 (6)	0	2 (11)	0	19 (22)	0	6 (11)	0	13 (10)	3 (2)	41 (13)	3 (1)
Any hepatic AE	0	0	2 (11)	0	8 (9)	0	3 (6)	2 (4)	5 (4)	2 (2)	18 (6)	4 (1)
ALT increased	0	0	1 (6)	0	6 (7)	0	1 (2)	0	3 (2)	1 (1)	11 (4)	1 (0.3)
Any Pulmonary AE	1 (6)	0	1 (6)	0	6 (7)	3 (4)	2 (4)	0	7 (5)	3 (2)	17 (6)	6 (2)
Pneumonitis	1 (6)	0	1 (6)	0	4 (5)	2 (2)	1 (2)	0	6 (5)	2 (2)	12 (4)	4 (1)
Other Select AE	0	0	1 (6)	0	3 (4)	0	3 (6)	0	8 (6)	2 (2)	15 (5)	2 (1)
Infusion-related reaction	0	0	1 (6)	0	3 (4)	0	3 (6)	0	5 (4)	0	12 (4)	0

Abbreviations: ALT: alanine aminotransferase, TSH: thyroid stimulating hormone
Source: Preliminary data, MDX1106-03. Clinical data cut-off date: 18-Mar-2013

7.1.2 Bevacizumab: Adverse Event Profile

In the initial Phase I and II clinical trials, four potential bevacizumab-associated safety signals were identified: hypertension, proteinuria, thromboembolic events, and hemorrhage. Additional completed Phase II and Phase III studies of bevacizumab as well as spontaneous reports have further defined the safety profile of this agent. Bevacizumab-associated adverse events identified in Phase III trials include congestive heart failure (CHF) primarily in metastatic breast cancer, gastrointestinal perforations, wound healing complications, and arterial thromboembolic events (ATE).

Hypertension: An increased incidence of hypertension has been observed in subjects treated with bevacizumab. Grade 4 and 5 hypertensive events are rare. Clinical sequela of hypertension are rare but have included hypertensive crisis, hypertensive encephalopathy, and

reversible posterior leukoencephalopathy syndrome (RPLS).[54,66] There is no information on the effect of bevacizumab in subjects with uncontrolled hypertension at the time of initiating bevacizumab therapy. Therefore, caution should be exercised before initiating bevacizumab therapy in these subjects. Monitoring of blood pressure is recommended during bevacizumab therapy. Optimal control of blood pressure according to standard public health guidelines is recommended for subjects on treatment with or without bevacizumab. Temporary interruption of bevacizumab therapy is recommended in subjects with hypertension requiring medical therapy until adequate control is achieved. If hypertension cannot be controlled with medical therapy, bevacizumab therapy should be permanently discontinued. Bevacizumab should be permanently discontinued in subjects who develop hypertensive crisis or hypertensive encephalopathy.

Proteinuria: An increased incidence of proteinuria has been observed in subjects treated with bevacizumab compared with control arm subjects. In the bevacizumab-containing treatment arms of clinical trials (across all indications), the incidence of proteinuria (reported as an adverse event) was up to 38% (metastatic CRC Study AVF2192g).[67] The severity of proteinuria has ranged from asymptomatic and transient events detected on routine dipstick urinalysis to nephrotic syndrome; the majority of proteinuria events have been Grade 1. NCI-CTC Grade 3 proteinuria was reported in up to 3% of bevacizumab-treated subjects, and Grade 4 in up to 1.4% of bevacizumab-treated subjects. The proteinuria seen in bevacizumab clinical trials was not associated with renal impairment and rarely required permanent discontinuation of bevacizumab therapy.

Bevacizumab should be discontinued in subjects who develop Grade 4 proteinuria (nephrotic syndrome). Subjects with a history of hypertension may be at increased risk for the development of proteinuria when treated with bevacizumab. There is evidence from the dose-finding, Phase II trials (AVF0780g, AVF0809s, and AVF0757g) suggesting that Grade 1 proteinuria may be related to bevacizumab dose. Proteinuria will be monitored by urinalysis, and urine protein: creatinine (UPC) ratio when necessary.

Thromboembolic Events: Both venous and arterial thromboembolic (TE) events, ranging in severity from catheter-associated phlebitis to fatal, have been reported in subjects treated with bevacizumab in the colorectal cancer trials, the recurrent glioblastoma trial and, to a lesser extent, in subjects treated with bevacizumab in NSCLC and breast cancer trials. Venous thromboembolic events (VTE) have also been observed in trials with bevacizumab and glioblastoma. To assess the overall risk of VTE associated with the use of bevacizumab, a systemic review and meta-analysis was performed and included prospective randomized controlled trials in which standard antineoplastic therapy was used with and without bevacizumab.[68] A total of 7,956 subjects with a variety of advanced solid tumors from 15 trials

were identified. Among the subjects treated with bevacizumab, the rates of all-grade and high-grade VTE were 11.9% and 6.3, respectively. Subjects treated with bevacizumab had a significantly increased risk of VTE compared with controls (RR 1.31). Since TE events are very common in GBM independent of treatment,[2] the relationship of thromboembolism to bevacizumab in this population is uncertain. Based on a Phase II clinical trial of bevacizumab with or without irinotecan in recurrent GBM, the rates of arterial thromboembolism were 2.4%-2.5% and venous thromboembolism were 3.6%- 8.9%.[60] The first incidence of VTE will therefore not constitute a DLT.

An increased incidence of arterial thromboembolic events (ATE) was observed in subjects treated with bevacizumab compared with those receiving control treatments. ATE includes cerebrovascular accidents, myocardial infarction, transient ischemic attacks (TIAs), and other ATE. The analysis of pooled data of 1,745 subjects from five randomized trials using bevacizumab combined with chemotherapy showed an increased risk of ATE (3.8% in treatment arm vs. 1.7% in the control arm) but not VTE.[69] Most ATE episodes described were myocardial or cerebrovascular events. Development of an ATE event was associated with a prior ATE event or age ≤ 65 years.

Gastrointestinal perforation: Subjects may be at increased risk for the development of gastrointestinal perforation and fistula when treated with bevacizumab and steroids or chemotherapy. Bevacizumab should be permanently discontinued in subjects who develop gastrointestinal perforation. A causal association of intra-abdominal inflammatory processes and gastrointestinal perforation to bevacizumab treatment has not been established. Nevertheless, caution should be exercised when treating subjects with intra-abdominal inflammatory processes with bevacizumab. A meta-analysis of 17 randomized controlled trials demonstrated a significantly increased risk of gastrointestinal perforation in subjects treated with bevacizumab compared to control medication.[70] The incidence was 0.9%, and the risks varied with tumor type, with colorectal cancer and renal cell cancer having the highest risk. In a Phase II clinical trial of bevacizumab with or without irinotecan for subjects with recurrent GBM, 2.1%-2.5% experienced a Grade 3 gastrointestinal perforation.[59,60]

Fistula: Bevacizumab use has been associated with serious cases of fistulae including events resulting in death. Fistulae in the GI tract are common (1%–10% incidence) in subjects with metastatic CRC, but uncommon (0.1%-1%) or rare (0.01%–0.1%) in other indications. In addition, fistulae that involve areas of the body other than the GI tract (e.g. tracheoesophageal, bronchopleural, urogenital, biliary) have been reported uncommonly (0.1%–1%) in subjects receiving bevacizumab in clinical studies and post-marketing reports. Events were reported at

various time points during treatment, ranging from 1 week to > 1 year following initiation of bevacizumab, with most events occurring within the first 6 months of therapy.

Wound healing complications: Wound healing complications such as wound dehiscence have been reported in subjects receiving bevacizumab. Most clinical trials with bevacizumab have required at least 28 days from any major surgery before starting treatment.[71] In a retrospective analysis of randomized trials in metastatic colorectal cancer, for a subset of subjects who had surgeries 28-60 days before initiating bevacizumab, the incidence of wound complications were low (1.3%),[72] indicating that the 28-day interval from colonic surgery might be appropriate. However, in the subset of subjects undergoing emergent surgery while on study, 13% of the subjects in the bevacizumab arm developed Grade 3 or Grade 4 postoperative wound complications compared to 3.4% of the subjects in the chemotherapy arm. Surgery in subjects currently receiving bevacizumab is not recommended. No definitive data are available to define a safe interval after bevacizumab exposure with respect to wound healing risk in subjects receiving elective surgery; however, the estimated half-life of bevacizumab is 21 days. Bevacizumab should be discontinued in subjects with severe wound healing complications. If subjects receiving treatment with bevacizumab require elective major surgery, it is recommended that bevacizumab be held for 4–8 weeks prior to the surgical procedure. Subjects undergoing a major surgical procedure should not begin or restart bevacizumab until 4 weeks after that procedure (in the case of high-risk procedures such as liver resection, thoracotomy, or neurosurgery, it is recommended that bevacizumab be restarted no earlier than 4 weeks after surgery). In a Phase II clinical trial of bevacizumab with or without irinotecan in subjects with recurrent GBM, Grade 3 or higher wound-healing complications were reported in 1.3-2.4%.[60]

1132 subjects treated with bevacizumab in a pooled database from eight Phase I, Phase II, and Phase III clinical trials in multiple tumor types (Genentech 2005). The hemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumor-associated hemorrhage (See below: Tumor-Associated Hemorrhage) and minor mucocutaneous hemorrhage.

Tumor-Associated Hemorrhage: Major hemorrhage has been observed primarily in subjects with NSCLC. Life-threatening and fatal hemoptysis was identified as a bevacizumab-related adverse event in NSCLC trials. These events occurred suddenly and presented as major or massive hemoptysis. GI hemorrhages, including rectal bleeding and melena have been reported in subjects with CRC, and have been assessed as tumor associated hemorrhages. Grades 1-4 tumor-associated hemorrhages were only very rarely seen in subjects with GBM (less than 4%).[60] Two of the five subjects who developed intracranial hemorrhage were anticoagulated at the time of the hemorrhage; both hemorrhages were Grade 1.

Mucocutaneous Hemorrhage: Across all bevacizumab clinical trials, mucocutaneous hemorrhage has been seen in 20%-40% of subjects treated with bevacizumab (Genentech 2005). These were most commonly NCICTC Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen. There have also been less common events of minor mucocutaneous hemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

Reversible Posterior Leukoencephalopathy Syndrome: There have been rare reports of bevacizumab-treated subjects developing signs and symptoms that are consistent with RPLS, a rare neurologic disorder that can present with the following signs and symptoms (among others): seizures, headache, altered mental status, visual disturbance, or cortical blindness, with or without associated hypertension. Brain imaging is mandatory to confirm the diagnosis of RPLS. In subjects who develop RPLS, treatment of specific symptoms, including control of hypertension, is recommended along with discontinuation of bevacizumab. The safety of reinitiating bevacizumab therapy in subjects previously experiencing RPLS is not known. In a Phase II clinical trial of bevacizumab with or without irinotecan in subjects with recurrent GBM, only one patient (1.3%) experienced serious reversible posterior leukoencephalopathy syndrome.[60]

Congestive heart failure: In clinical trials CHF was observed in all cancer indications studied to date, but predominantly in subjects with metastatic breast cancer. In the Phase III clinical trial of metastatic breast cancer (AVF2119g), 7 (3%) bevacizumab-treated subjects experienced CHF, compared with two (1%) control arm subjects.[73] These events varied in severity from asymptomatic declines in left ventricular ejection fraction (LVEF) to symptomatic CHF requiring hospitalization and treatment. All the subjects treated with bevacizumab were previously treated with anthracyclines (doxorubicin cumulative dose of 240-360 mg/m2). Many of these subjects also had prior radiotherapy to the left chest wall. Most of these subjects showed improved symptoms and/or left ventricular function following appropriate medical therapy.[73] No information is available on subjects with preexisting CHF of New York Heart Association (NYHA) Class II-IV at the time of initiating bevacizumab therapy, as these subjects were excluded from clinical trials.

Adverse events in GBM studies from FDA labeling information: Bevacizumab is commercially available and FDA approved for subjects with recurrent glioblastoma. For further details, see the bevacizumab FDA labeling information available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125085s0169lbl.pdf. All adverse events were collected in 163 subjects enrolled in a non-comparative Phase II study who either received bevacizumab alone or bevacizumab plus irinotecan.[60] All subjects received prior

radiotherapy and temozolomide. Bevacizumab was administered at 10 mg/kg every 2 weeks alone or in combination with irinotecan. Bevacizumab was discontinued due to adverse events in 4.8% of subjects treated with bevacizumab alone.

In subjects receiving bevacizumab alone (N=84), the most frequently reported adverse events of any grade were infection (55%), fatigue (45%), headache (37%), hypertension (30%), epistaxis (19%) and diarrhea (21%). Of these, the incidence of Grade ≥ 3 adverse events was infection (10%), fatigue (4%), headache (4%), hypertension (8%) and diarrhea (1%). Two deaths on study were possibly related to bevacizumab: one retroperitoneal hemorrhage and one neutropenic infection. In subjects receiving bevacizumab alone or bevacizumab plus irinotecan (N=163), the incidence of bevacizumab-related adverse events (Grade 1−4) were bleeding/hemorrhage (40%), epistaxis (26%), CNS hemorrhage (5%), hypertension (32%), venous thromboembolic event (8%), arterial thromboembolic event (6%), wound-healing complications (6%), proteinuria (4%), gastrointestinal perforation (2%), and RPLS (1%). The incidence of Grade 3−5 events in these 163 subjects were bleeding/hemorrhage (2%), CNS hemorrhage (1%), hypertension (5%), venous thromboembolic event (7%), arterial thromboembolic event (3%), wound-healing complications (3%), proteinuria (1%), and gastrointestinal perforation (2%).

7.2 Definitions

7.2.1 Adverse Events

An ***Adverse Event (AE)*** is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

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

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

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

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

7.2.2 Serious Adverse Events

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

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Potential drug induced liver injury (DILI) is also considered an important medical event.
- Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.
- Although pregnancy, overdose, cancer, and potential drug induced liver injury (DILI) are not always serious by regulatory definition, these events must be handled as SAEs.
- Any component of a study endpoint that is considered related to study therapy (e.g., death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported) should be reported as SAE

NOTE:
The following hospitalizations are not considered SAEs in BMS clinical studies:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure

- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anti-cancer therapy in the absence of any other SAEs

Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs. Potential drug induced liver injury is defined as:

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

AND

- Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

AND

- No other immediately apparent possible causes of AST/ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

7.3 Reporting Procedures for All Adverse Events

All participating investigators will assess the occurrence of AEs throughout the subject's participation in the study starting with day 1 of treatment. Subjects will be followed for toxicity for 100 days after treatment has been discontinued or until death, whichever occurs first. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

The investigator is responsible for ensuring that all adverse events observed by the investigator or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's case report form, subject's medical records, and/or any other

institutional requirement. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study), requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event.

The investigator will provide the following for all adverse events:

- Description of the event
- Term that defines seriousness for the particular event, e.g., Hospitalization or Important Medical Event
- Date of onset and resolution
- Grade of toxicity
- Attribution of relatedness to each of the investigational agents, in this study nivolumab
- Action taken with each agent as a result of the event
- Outcome of event

In this study, descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 available at http://ctep.cancer.gov will be utilized for AE reporting.

Investigative sites will report adverse events to their respective IRB according to the local IRB's policies and procedures in reporting adverse events.

7.4 Reporting Procedures for Serious Adverse Events

Serious adverse events that occur beginning with the signing of the informed consent form, during treatment, or within 100 days of the last dose of treatment must be reported to the Principal Investigator.

SAEs will be reported promptly to the Sponsor-Investigator David Peereboom M.D. or his designated study coordinator once the investigator determines that the event meets the protocol definitions of an SAE.

Once an investigator becomes aware that an SAE has occurred in a study patient, the investigator must report the information to the Sponsor-Investigator David Peereboom M.D. or his designated study coordinator within 24 hours

Investigative sites will report serious adverse events to their respective IRB according to the local IRB's policies and procedures in reporting serious adverse events.

7.4.1 Bristol-Myers Squibb Company Serious Adverse Event 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 Pregnancy Surveillance Form.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

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

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

All SAEs should be followed to resolution or stabilization.

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

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.

MedWatch SAE forms should be sent to the FDA at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

All SAEs should simultaneously be faxed or e-mailed to BMS at:
Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

Reporting Serious Adverse Events to the IRB/IEC: It is the Investigator's responsibility to report SAEs to the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) according to the requirements of the governing IRB/IEC.

- Worldwide.Safety@bms.comaepbusinessprocess@bms.com

7.4.2 FDA Reporting

The Cleveland Clinic Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. In accordance with 21 CFR 312.32, the Cleveland Clinic Principal Investigator is responsible for notifying the FDA of SAEs that are serious, unexpected (not listed in the Investigator Brochure) and judged to be related (i.e., possible, probable, definite) to the study drug. Events meeting the following criteria need to be submitted to the FDA as Expedited IND Safety Reports.

7 Calendar Day IND Safety Report
Any unexpected fatal or life-threatening suspected adverse event represents especially important safety information and, therefore, must be reported more rapidly to FDA (21 CFR 312.32(c)(2)). Any unexpected fatal or life-threatening suspected adverse event must be reported to FDA no later than 7 calendar days after the Cleveland Clinic Investigator's initial receipt of the information (21 CFR 312.32(c)(2)). Cleveland Clinic Principal Investigator will complete a Medwatch Form FDA 3500A and notify the FDA by telephone or facsimile transmission.

15 Calendar Day IND Safety Report
The timeframe for submitting an IND safety report to FDA and all participating investigators is no later than 15 calendar days after the Cleveland Clinic Principal Investigator determines that the suspected adverse event or other information qualifies for reporting (21 CFR 312.32(c)(1)). This includes any serious, unexpected adverse events considered reasonably or possibly related to the investigational agent and that are not life-threatening or fatal. The Cleveland Clinic Principal Investigator will complete a Medwatch Form FDA 3500A and notify the FDA by telephone or facsimile transmission. If FDA requests any additional data or information, the Cleveland Clinic Principal Investigator must submit it to FDA as soon as possible, but no later than 15 calendar days after receiving the request (21 CFR 312.32(c)(1)(v)).

Follow-up IND Safety Report
Any relevant additional information that the Cleveland Clinic Principal Investigator obtains that pertains to a previously submitted IND safety report must be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)). The Cleveland Clinic Principal Investigator will maintain records of its efforts to obtain additional information.

Reporting Serious Problems to FDA
Medwatch Form FDA 3500A:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm
Telephone: 1-800-332-1088
Fax: 301-796-9849

The fax cover sheet should note that this report will also be submitted formally in triplicate to the IND as an amendment per 21 CFR 312.32 (i.e. a formal paper submission to the Beltsville address).

IND Annual Reports
A summary of all IND safety reports submitting during the previous year will be reported to the FDA in the annual report by the Cleveland Clinic principal investigator, as holder of the IND.A copy will be sent to BMS.

SAEs and OnCore – added to OnCore™ Database
- All SAEs will be entered into OnCore.
- A copy of the SAE form(s) submitted to the sponsor-investigator is also uploaded into Oncore.

7.5 Data Safety and Toxicity Committee

It is the responsibility of each site PI to ensure that ALL SAEs occurring on this trial (internal or external) are reported to the Case Comprehensive Cancer Center's Data and Safety Toxicity Committee. This submission is simultaneous with their submission to the sponsor and/or other regulatory bodies.

The sponsor-investigator is responsible for submitting an annual report to the DSTC as per CCCC Data and Safety Monitoring Plan.

7.6 Data and Safety Monitoring Plan (DSMP)

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI guidelines.

8.0 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.0.

8.1 Nivolumab

Name of agent: Nivolumab

Nomenclature

Research Name: BMS-936558

Generic Name: Nivolumab

Description of Nivolumab

Nivolumab (also referred to as BMS-936558 or MDX1106) is a fully human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligands 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens. The clinical study product is a sterile solution for parenteral administration.

Drug Substance Chemistry

Nivolumab is a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains. Nivolumab has a molecular weight of 146,221 daltons. It is a clear to opalescent, colorless to pale yellow liquid, which may contain light (few) particulates. The clinical study product is a sterile solution for parenteral administration.

Drug Product

Please refer to Investigational Brochure in Appendix.

Container/Closure

Nivolumab has a concentration of 10mg/mL and is provided in a 10mL vial. Ten or five vials are provided in a carton.

Storage and Handling of Pharmaceutical Form

- Store at 2-8°C (36-46°F), protect from light, freezing, and shaking.
- If any temperature excursions are encountered during storage, please report these to BMS for assessment via the Temperature Excursion Response Form.
- As with all injectable drugs, care should be taken when handling and preparing nivolumab. Whenever possible, nivolumab should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents applying aseptic technique.
- Partially used vials should be disposed at the site following procedures for the disposal of anticancer drugs.

After final drug reconciliation, unused nivolumab vials should be disposed at the site following procedures for the disposal of anticancer drugs.

Use Time/Stability

Due to parameters surrounding the use time of Nivolumab, the time of preparation should be noted in the Pharmacy Source documents [accountability logs] or in study files as required for investigator sponsored research [FDA and GCP]

The administration of BMS-936558-01 injection prepared for dosing nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 20 hours in a refrigerator at under refrigeration conditions (2°-°-8°C (, 36°-46°F) and used within 4for up to 24 hours, and a maximum of 4 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and under room light. The maximum 4-hour period under room temperature and room light conditions for undiluted and diluted solutions of BMS-936558-01 injection in the IV bag should be inclusive of the includes the product administration period.

Drug Preparation and Administration:

1. Visually inspect the drug product solution for particulate matter and discoloration prior to administration. Discard if solution is cloudy, if there is pronounced discoloration (solution may have a pale-yellow color), or if there is foreign paticulate matter other than a few translucent-to-white, amorphous particles.

   *Note: Mix by **gently** inverting several times. **Do not** shake.*
2. Aseptically withdraw the required volume of nivolumab solution into a syringe, and dispense into an IV. bag. If multiple vials are needed for a subject, it is important to use a separate sterile syringe and needle for each vial to prevent problems such as dulling of needle tip, stopper coring, repeated friction of plunger against syringe barrel wall. **Do not** enter into each vial more than once. **Do not** administer study drug as an IV push or bolus injection
3. Add the appropriate volume of 0.9% Sodium Chloride Injection solution or 5% Dextrose Injection solution. *It is acceptable to add nivolumab solution from the vials into an appropriate pre-filled bag of diluent.*

   Note: Nivolumab infusion concentration must be at or above the minimum allowable concentration of 0.35 mg/mL [IBV13 Addendum Section 3.2.2]

   Note: It is not recommended that so-called "channel" or tube systems are used to transport prepared infusions of nivolumab.
4. Attach the IV bag containing the nivolumab solution to the infusion set and filter.
5. At the end of the infusion period, flush the line with a sufficient quantity of approved diluents.

Drug Accountability
The investigator or designated study personnel are responsible for maintaining accurate dispensing records of the study drug. All study drugs must be accounted for, including study drug accidentally or deliberately destroyed. Under no circumstances will the investigator allow the investigational drug to be used other than as directed by the protocol. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

Drug Destruction
At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.[22]

8.2 Bevacizumab

Form

Bevacizumab is a clear to slightly opalescent, colorless to pale brown, sterile liquid concentrate for solution for intravenous (IV) infusion. Vials contain bevacizumab with phosphate, trehalose, polysorbate 20, and sterile water for injection (SWFI), USP. Vials contain no preservative and are suitable for single use only. This agent is commercially available and manufactured by Genentech.

Storage and Stability

According to guidelines specified in the package insert.

Preparation

According to guidelines specified in the package insert.

Administration

Bevacizumab is to be administered according to institutional standards.

The dose of bevacizumab is 10 mg/kg IV for subjects on Arm A and 3 mg/kg for subjects on Arm B. Bevacizumab is administered on Days 1, 15 of each 4 week cycle. A window of +/- 3

day for bevacizumab dosing is acceptable but bevacizumab doses must be at least 12 days apart.

Accountability
The investigator or designated study personnel are responsible for maintaining accurate dispensing records of bevacizumab which are to include start time of infusion, stop time of infusion, total volume.

9.0 STUDY PARAMETERS AND CALENDAR

9.1 Study Parameters

9.1.1 Screening Evaluation

Screening studies and evaluations will be used to determine the eligibility of each subject for study inclusion. All evaluations must be completed $\leq$ 14 days prior to administration of protocol therapy, except where otherwise noted.

9.1.2 Treatment Period

Enrolled subjects will randomize to Arm A (nivolumab + standard bevacizumab) and Arm B (nivolumab and low dose bevacizumab). Treatment is every 2 weeks and will continue until evidence of disease progression as determined by iRANO criteria, unacceptable toxicity, or death.
In view of the Covid 19 crisis, all in person visits can be substituted for virtual visit. All nursing toxicity checks can be performed over the phone rather than in person.

9.2 Study Calendar

Table 9.2.1 Study Calendar: Assessments prior to, during, and after therapy (Both Arms A and B):

	Screening w/in 28 days unless otherwise indicated	All Cycles – both odd and even Day 1 Week 1, then every 2 weeks (± 4 days) – unless otherwise indicated	Follow-up #1 - [z] Follow-up #2 - [aa] Survival - [bb]
Informed Consent	X[a]		
Inclusion/Exclusion Criteria	X[b]		
Medical History	X		
Tumor Tissue Availability	X[d, h]		
MGMT Status	X[c]		
Physical & Neurological Exam	X (within 14 days) [l]	X (not required D1 Wk 1) [l, cc]	X [l]
Vital Signs	X[f]	X[f, cc]	X[f]
Performance Status (KPS)	X[g]	X[N, cc]	X
Height and Weight	X[e]	X[N]	X
Adverse Event Assessment	X[g]	Continuously	X
Concomitant Medication Collection & Review	X[g]	X	X
Steroid Documentation &	X[g]	X	X

Review			

CBC w/ differential	X [g]	X [o]	X
CMP	X [g]	X [o]	X
LDH	X [g]	X [o]	X
Magnesium	X [g]	X [o]	
Amylase	X [g]	X [o]	
Lipase	X [g]	X [o]	
TSH	X [g]	X [o,p]	X
FT4	X [g]	X [o,p]	
FT3	X [g]	X [o,p]	
HBsAg, HCV	X [g]		
Cytokine/chemokine/PBMC Correlative Studies	X [g, h]	X [h, u]	
Urinalysis – dipstick	X [g]	X [r]	
HCG – serum (WOCBP only)	X [g]	X s	X
12-Lead EKG	X [g]	X [t]	X [t]
Tumor Assessments	X [l]	X [j,dd]	X [y]
NANO scale		X [v]	

Administer Study Treatment		Within 3 working days of randomization: Nivolumab and Bevacizumab administered every 2 weeks +/- 2days X [bb]	
Survival Status			X [w]

a. Study allows for re-enrollment of a subject that has discontinued the study as a pre-treatment failure.
b. Inclusion/Exclusion criteria should be assessed at screening and confirmed prior to randomization.
c. If available
d. Archival or fresh tissue taken at any point prior to study treatment. Up to 10 unstained slides of 5 microns thickness or block tissue will be required.
e. Weight (Day 1 Wk 1, then every 4 weeks); height (only at baseline)
f. BP, HR, temperature and respiratory rate
g. Within 14 days prior to randomization
h. See Appendix 8
i. Within 21 days of randomization
j. Every 8 weeks (+/- 1 week)
k. Perform every 28 days on D1 of each cycle (+/- 4 days)
l. Includes cardiovascular, neurological and abdominal exams; D1 of each cycle
m. Within 72 hours prior to first dose
n. Day 1 of each cycle
o. Complete prior to each dose through Wk 21, then every 4 weeks (Day 1 of each cycle).
p. After C1D1 – TSH, FT3, FT4 are done every 8 weeks (+/- 1 week)
q. blank
r. Dipstick within 72 hours prior to each bevacizumab dose. Subjects with 4+ (300-1000) proteinuria readings must undergo further assessment with a 24 hour urine collection prior to being dosed.
s. Every 4 weeks (+/- 1 week)
t. As clinically indicated
u. Obtain at week 4, week 8, and then at every MRI visit until progression
v. Completed by study physician prior to dosing Wk 1 Day 1, and then with each MRI (must be done before MRI is reviewed with the subject)
w. Repeat labs at follow-up visit #2 if study drug related toxicities persist
x. May be obtained through a phone call or clinic visit
y. Only for subjects who did not progress while on study therapy, including subjects who start subsequent anticancer therapy. Tumor assessments will not be collected for subjects who are lost to follow or withdraw consent.

z. Subjects must be followed for at least 100 days after the last dose of study therapy. Follow-up visit #1 occurs approximately 35 days (+/- 1 week) after last dose or coinciding with the date of discontinuation so long as the date of discontinuation is greater than 35 days after last dose.
aa. Follow-up visit #2 occurs approximately 80 days (+/- 1 week) after F/U #1
bb. +/- 2 days
cc. In view of covid-19 crisis, virtual visits will be allowed and the need for physical exam as long as patient is asymptomatic will be waived.

dd. Patients who remain on study after 3 years , MRIs to be done every 12 weeks (+/- 1 week)

Survival visits/calls = every 3 months from

10.0 MEASUREMENT OF EFFECT

10.1 RANO Criteria

Definitions of Tumor Response will be based on RANO criteria. There is an exploratory analysis using the iRANO criteria.[65] Please refer to Appendix 2.

11.0 DATA REPORTING / REGULATORY CONSIDERATIONS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

11.1 Data Reporting

The Advarra EDC™ and OnCore™ databases will be utilized, as required by the Case Comprehensive Cancer Center and Cleveland Clinic, to provide data collection for both accrual entry and trial data management. Advarra EDC and OnCore™ are Clinical Trials Management Systems housed on secure servers. Access to data through Advarra EDC and OnCore™ is restricted by user accounts and assigned roles. Once logged into the Advarra EDC or OnCore™ system with a user ID and password, Advarra EDC™ and OnCore™ define roles for each user which limits access to appropriate data. Applications for user accounts can be obtained by contacting the OnCore™ Administrator at OnCore-registration@case.edu for OnCore™ access, and taussigoncore@ccf.org for Advarra EDC™ access.

Advarra EDC™ is designed with the capability for study setup, activation, tracking, reporting, data monitoring and review, and eligibility verification. When properly utilized, Advarra EDC™ is 21 CFR 11 compliant. This study will utilize electronic Case Report Form completion in the Advarra EDC™ database. A calendar of events and required forms are available in Advarra EDC™.

11.2 Regulatory Considerations

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

11.2.1 Written Informed consent

Provision of written informed consent must be obtained prior to any study-related procedures. The Principal Investigator will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the study as well as the subject's financial responsibility. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and be allowed time to consider the information provided.

The original, signed written Informed Consent Form must be kept with the Research Chart in conformance with the institution's standard operating procedures. A copy of the signed written

Informed Consent Form must be given to the subject. Additionally, documentation of the consenting process should be located in the research chart.

11.2.2 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), a subject must sign an authorization to release medical information to the sponsor and/or allow the sponsor, a regulatory authority, or Institutional Review Board access to subject's medical information that includes all hospital records relevant to the study, including subjects' medical history.

11.2.3 Retention of records

The Principal Investigator of The Case Comprehensive Cancer Center supervises the retention of all documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence for as long as needed to comply with local, national and international regulations. No records will be destroyed until the Principal Investigator confirms destruction is permitted.

11.2.4 Audits and inspections

Authorized representatives of the sponsor, a regulatory authority, an Independent Ethics Committee (IEC) or an Institutional Review Board (IRB) may visit the site to perform audits or inspections, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. For multi-center studies, participating sites must inform the sponsor-investigator of pending audits.

11.2.5 Data Safety and Monitoring Plan

This protocol will adhere to the policies of the Case Comprehensive Cancer Center Data and Safety Monitoring Plan in accordance with NCI regulations.

12.0 STATISTICAL CONSIDERATIONS

STATISTICAL CONSIDERATIONS

This section outlines the statistical analysis strategy and procedures for the study. If, after the study has begun, changes are made to primary and/or key secondary hypotheses, or the

statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, along with an explanation as to when and why they occurred, will be listed in the Clinical Study Report (CSR) for the study. Post hoc exploratory analyses will be clearly identified in the CSR. No separate Statistical Analysis Plan (SAP) for the primary, secondary and exploratory endpoints will be issued for this study.

12.1 Statistical Analysis Plan Summary

This section contains a brief summary of the statistical analyses for this trial. This trial includes randomization of accrual to two experimental treatment arms: nivolumab plus bevacizumab at standard dosing (Arm A); and nivolumab plus bevacizumab at reduced dosing (Arm B). The outcome of each treatment arm will be assessed separately relative to appropriate historical controls.
The study consists of two arms to evaluate the efficacy and tolerability and safety profile of nivolumab in combination with reduced or standard dose bevacizumab in subjects with recurrent glioblastoma (in first recurrence). For this purpose, 90 eligible and evaluable subjects will be randomized in a 1:1 ratio to arm A (nivolumab plus standard bevacizumab) or arm B (nivolumab plus reduced dose bevacizumab). To facilitate robust data for assessing all objectives and generating future hypotheses, stratified randomization will be implemented in REDCap. The design will balance treatment assignments based on four stratification factors known to be prognostic for clinical outcomes. The stratification factors include: age (<=65 versus > 65), KPS (>=800 versus <800), Methylation Status (Not Methylated versus Methylated), Surgery Type (Biopsy Only, Partial Resection, Complete Resection/Near Complete Resection).

The primary and secondary goals of the trial are to evaluate the safety and efficacy of the two therapies and to obtain a preliminary assessment of whether or not they differ with respect to outcome.

The total sample size of 90 eligible and evaluable subjects randomized 1:1 to the two therapies is recommended to provide adequate statistical power to describe the efficacy and toxicity profiles of the two treatment arms.

Using the BELOB trial, EORTC 26101 trial outcomes as historical benchmarks, the primary measure of efficacy for the current study will be the 12 month overall survival rate (OS-12)[47,48]. OS-12 was approximately 45% in the BELOB and EORTC 26101 trials. The one-sample log-rank test will be applied to outcomes observed for each arm individually to test the hypothesis that OS has been improved beyond the null 12-month survival rate of 45%. With N=45 subjects per arm, a one-sided test provides power=0.80 to detect survival rate of 58% at 12-months following treatment at the 0.10 significance level. Statistical calculations were implemented with PASS version 15.0.5.

Although each treatment arm will be evaluated for efficacy independently they will also be compared in order to get a preliminary read on whether there is a signal that one may be superior to the other. The sample size of N=45 subjects per treatment arm provides power=0.80 to detect a hazard ratio of 0.498 between the two study arms using a two-sided stratified log-rank test. Statistical calculations were implemented with PASS version 15.0.5.

With 45 subjects per arm the risk that a particular type and/or grade of adverse event will occur in a particular arm will be estimable using an exact 95% confidence interval that has a maximum half-width of 15%. For example, if 5 adverse events are observed (11%) the corresponding 95% confidence interval will be 4%-24%; if 20 are observed (44%) it will be 30%-60%. In addition, the likelihood of observing at least one adverse event of a particular type and/or grade is ≥90% even if the risk of such an event is only 5%. Although excessive toxicity is not expected, adverse events will be monitored continuously and the following table will be used as a guide for whether or not a formal review, possibly leading to early termination, should be considered.

		Cumulative Likelihood of Exceeding the AE Threshold if the Risk of an Event is			
No. Subjects	**Consider Review if the Cumulative No. Tx-Related Grade ≥3 AEs Exceeds**	**10%**	**20%**	**30%**	**40%**
50%					
1-10	4	<1%	3%	15%	37%
62%					
11-20	7	<1%	5%	27%	62%
88%					
21-30	10	<1%	6%	36%	76%	96%

12.1.1 Efficacy Analysis

The primary and key secondary endpoints, primary analysis population, and statistical methods that will be employed for the efficacy analyses are discussed in detail in the following sections**.**

The primary hypothesis of efficacy will be evaluated independently in each cohort.

12.1.2 Safety Analysis

Adverse events will be graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. All subjects who receive any amount of nivolumab will be evaluable for toxicity. The All-Subjects-as-Treated population will be employed for safety analyses. Immune related adverse experiences are pre-specified as Events of Clinical Interest (Section 7.6.2).

12.2 Statistical Analysis Plan

12.2.1 Responsibility for Analyses
The statistical analysis of the data obtained from this study will be the responsibility of the study responsible biostatistician.

This trial is being conducted as an open-label study, i.e., subjects, investigators, and SPONSOR personnel will be aware of subject treatment assignments after each subject is enrolled and treatment is assigned.

12.2.1 Efficacy Endpoints

All subjects within each arm that have been randomized will serve as the primary population for the analysis of the efficacy data in this study. Two supportive analyses of the primary and selected secondary efficacy endpoints will also be conducted. The first supportive analysis will include all subjects in the primary population for analysis who have a post baseline scan OR discontinue the trial due to progressive disease/drug related AE. The second analysis will be conducted using the intention to treat (ITT) population, defined as all randomized subjects. Subjects will be included in the arm to which they are randomized for the analysis of efficacy data.

Efficacy endpoints that will be evaluated for are listed below, followed by the descriptions of the derivations of selected endpoints.

The primary efficacy endpoint for both cohorts is OS-12, defined as the proportion of subjects in the analysis population who remain alive for at least twelve months following initiation of study therapy. Response for the primary analysis will be determined by the investigator assessment, and a confirmation assessment is required per RANO.[74]

Secondary efficacy endpoints include: (1) overall survival (OS); (2) ORR defined as the proportion of subjects in the analysis population who have complete response (CR) or partial response (PR) using RANO criteria as well as duration of response, defined as time from first RANO response to disease progression in subjects who achieve a PR or better; (3) progression-free survival (PFS), defined as the time from allocation to the first documented disease progression according to RANO or death due to any cause, whichever occurs first; and (4) progression-free survival at six months defined as the proportion of subjects in the analysis population who remain progression-free for at least six months following initiation of study therapy. Analyses of ORR, duration of response, and PFS will be conducted using RANO criteria, in which a confirmation assessment of disease progression must be obtained at least 4 weeks after the initial disease assessment indicating progressive disease.

Nominal p –values may be computed for efficacy analyses as a measure of strength of association between the endpoint and the treatment effect rather than formal tests of hypotheses. Unless otherwise stated, all statistical tests will be conducted at α=0.05 (2-sided) level.

Efficacy will be evaluated separately in each cohort. For PFS endpoint, Kaplan-Meier (KM) curves and median estimates from the KM curves will be provided as appropriate. Subjects without efficacy evaluation data or without survival data will be censored at Day 1. Participants without measurable disease will not be included in the analysis of ORR.

12.2.2 Safety Endpoints

All subjects who receive at least one dose of study treatment will be included in the safety data analysis. At least one laboratory or vital sign measurement obtained subsequent to at least one dose of study treatment is required for inclusion in the analysis of each specific parameter. To assess change from baseline, a baseline measurement is also required.

Safety analyses will be performed in all treated subjects. Descriptive statistics of safety will be presented using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 by treatment group. All on-study AEs, treatment-related AEs, SAEs, and treatment-related SAEs will be tabulated using worst grade per NCI CTCAE v 4.03 criteria by system organ class and preferred term. On-study lab parameters including

hematology, chemistry, liver function, and renal function will be summarized using worst grade NCI CTCAE v 4.03 criteria.

Adverse experiences (specific terms as well as system organ class terms) and predefined limits of change in laboratory, and vital sign parameters that are not pre-specified as events of interest will be summarized with descriptive statistics (counts, percentage, mean, standard deviation, etc.).

Continuous measures such as changes from baseline in laboratory, and vital signs parameters that are not pre-specified as events of interest will be summarized using descriptive statistics (mean, standard deviation, etc.) for baseline, on-treatment, and change from baseline values.

12.2.3 Analysis of Demographics and Baseline Characteristics
Baseline characteristics will be assessed by the use of tables and/or graphs for each cohort separately. No statistical hypothesis tests will be performed on these characteristics. The number and percentage of subjects screened, randomized, the primary reasons for screening failure, and the primary reason for discontinuation will be displayed. Demographic variables (e.g., age, gender), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized by treatment either by descriptive statistics or categorical tables.

12.2.4 Analysis of Immunocorrelative Data
Longitudinal analyses of PBMC immune response kinetics and circulating cytokines to nivolumab and bevacizumab therapy will be presented graphically and descriptively at each time point. Changes in the magnitude of the response relative to pre-treatment after nivolumab plus bevacizumab therapy will be summarized descriptively. Changes in response between pre-treatment and prior to initiation of cycle 3 of nivolumab plus bevacizumab therapy will be assessed using the Wilcoxon signed-rank test.

12.2.5 Power and Sample Size
The primary and secondary goals of the trial are to evaluate the safety and efficacy of the two therapies and to obtain a preliminary assessment of whether or not they differ with respect to outcome.

The total sample size of 90 eligible and evaluable subjects randomized 1:1 to the two therapies is recommended to provide good statistical power to describe the efficacy and toxicity profiles of the two treatment arms.

Using the BELOB trial, EORTC 26101 trial outcomes as historical benchmarks, the primary measure of efficacy for the current study will be the 12 month overall survival rate (OS-12). OS-12 was approximately 45% in the BELOB and EORTC 26101 trials.

The one-sample log-rank test will be applied to outcomes observed for each arm individually to test the hypothesis that OS has been improved beyond the null 12-month survival rate of 45%. With N=45 subjects per arm, a one-sided test provides power=0.80 to detect survival rate of 58% at 12-months following treatment at the 0.10 significance level. Statistical calculations were implemented with PASS version 15.0.5.

Although each treatment arm will be evaluated for efficacy independently they will also be compared in order to get a preliminary read on whether there is a signal that one may be superior to the other. The sample size of N=45 subjects per treatment arm provides power=0.80 to detect a hazard ratio of 0.498 between the two study arms using a two-sided stratified log-rank test. Statistical calculations were implemented with PASS version 15.0.5.

With 45 subjects per arm, the risk that a particular type and/or grade of adverse event will occur in a particular arm will be estimable using an exact 95% confidence interval that has a maximum half-width of 15%. For example, if 5 adverse events are observed (11%) the corresponding 95% confidence interval will be 4%-24%; if 20 are observed (44%) it will be 30%-60%. In addition, the likelihood of observing at least one adverse event of a particular type and/or grade is $\geq$90% even if the risk of such an event is only 5%. Although excessive toxicity is not expected, adverse events will be monitored continuously and the following table will be used as a guide for whether or not a formal review, possibly leading to early termination, should be considered.

No. Subjects	Consider Review if the Cumulative No. Tx-Related Grade ≥3 AEs Exceeds	Cumulative Likelihood of Exceeding the AE Threshold if the Risk of an Event is 10%	20%	30%	40%
50%					
1-10	4	<1%	3%	15%	37%
62%					
11-20	7	<1%	5%	27%	62%
88%					
21-30	10	<1%	6%	36%	76%
96%					

As can be seen the likelihood is small (<1% to 6%) of instituting a formal review if the level of serious toxicity is low (≤20%), moderate to high (15-76%) if significant toxicity is similar to the ≥26% grade 3/4 toxicity rate seen in subjects treated with single agent bevacizumab on the BELOB trial· or the 39% treatment related SAE rate seen in subjects treated with bevacizumab+lomustine in the EORTC 26101 trial***need to add reference no.***, and high (62-96%) if the risk of serious toxicity is ≥50%.

13.0 Ethical Considerations

13.1 Good Clinical Practice

This study will be conducted in accordance with Good Clinical Practice (GCP), as defined by the International Conference on Harmonisation (ICH) and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50).
The study will be conducted in compliance with the protocol. The protocol and any amendments and the subject informed consent will receive Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval/favorable opinion prior to initiation of the study.

All potential serious breaches must be reported to BMS immediately. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.
Personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks.
This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment).

13.2 Institutional Review Board/Independent Ethics Committee

Before study initiation, the investigator must have a written and dated approval/favorable opinion from the IRB/IEC for the protocol, consent form, subject recruitment materials (e.g., advertisements), and any other written information to be provided to subjects.
The investigator or BMS should also provide the IRB/IEC with a copy of the Investigator Brochure or product labeling information to be provided to subjects and any updates.
The investigator or BMS should provide the IRB/IEC with reports, updates and other information (e.g., expedited safety reports, amendments, and administrative letters) according to regulatory requirements or institution procedures.

Informed Consent

Investigators must ensure that subjects are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate.
In situations where consent cannot be given to subjects, their legally acceptable representatives are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which the subject volunteers to participate.
BMS will provide the investigator with an appropriate (i.e., Global or Local) sample informed consent form which will include all elements required by ICH, GCP and applicable regulatory requirements. The sample informed consent form will adhere to the ethical principles that have their origin in the Declaration of Helsinki.

Investigators must:

1) Provide a copy of the consent form and written information about the study in the language in which the subject is most proficient prior to clinical study participation. The language must be non-technical and easily understood.

2) Allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study.
3) Obtain an informed consent signed and personally dated by the subject or the subject's legally acceptable representative and by the person who conducted the informed consent discussion.
4) Obtain the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects, prior to the beginning of the study, and after any revisions are completed for new information.
5) If informed consent is initially given by a subject's legally acceptable representative or legal guardian, and the subject subsequently becomes capable of making and communicating his or her informed consent during the study, consent must additionally be obtained from the subject.
6) Revise the informed consent whenever important new information becomes available that is relevant to the subject's consent. The investigator, or a person designated by the investigator, should fully inform the subject or the subject's legally acceptable representative or legal guardian, of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented.

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules applicable to regulatory requirements, the subjects' signed ICF and, in the US, the subjects' signed HIPAA Authorization.

The consent form must also include a statement that BMS and regulatory authorities have direct access to subject records.

Subjects unable to give their written consent (e.g., stroke or subjects with or severe dementia) may only be enrolled in the study with the consent of a legally acceptable representative. The subject must also be informed about the nature of the study to the extent compatible with his or her understanding, and should this subject become capable, he or she should personally sign and date the consent form as soon as possible. The explicit wish of a subject who is unable to give his or her written consent, but who is capable of forming an opinion and assessing information to refuse participation in or to be withdrawn from, the clinical study at any time should be considered by the investigator.

The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

Case 1317
Protocol Version 10/24/2017
Protocol Amendment 1 05/03/2018
Protocol Amendment 2 05/21/2018
Protocol Amendment 3 01/06/2020
Protocol Amendment 4 03/20/2020
Protocol Amendment 5 07/14/2021

14.0 REFERENCES

1. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009;10:459-66.
2. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987-96.
3. Sampson JH, Aldape KD, Archer GE, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. Neuro-oncology 2011;13:324-33.
4. Sampson JH, Heimberger AB, Archer GE, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J Clin Oncol 2010;28:4722-9.
5. Liau LM, Prins RM, Kiertscher SM, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clinical cancer research : an official journal of the American Association for Cancer Research 2005;11:5515-25.
6. Prins RM, Soto H, Konkankit V, et al. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. Clin Cancer Res 2011;17:1603-15.
7. Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. Cancer Res 2004;64:4973-9.
8. Hussain SF, Kong LY, Jordan J, et al. A novel small molecule inhibitor of signal transducers and activators of transcription 3 reverses immune tolerance in malignant glioma patients. Cancer research 2007;67:9630-6.
9. Heimberger AB, Bigner DD, Sampson JJ. Biological principles of brain tumor immunotherapy. In: Liau LM, Becker DP, Cloughesy T, Bigner DD, eds. Brain tumor immunotherapy. Totowa, N.J.: Humana Press; 2000.
10. Hussain SF, Yang D, Suki D, Aldape K, Grimm E, Heimberger AB. The role of human glioma-infiltrating microglia/macrophages in mediating antitumor immune responses. Neuro-oncology 2006;8:261-79.
11. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 2007;13:84-8.
12. Heimberger AB, Abou-Ghazal M, Reina-Ortiz C, et al. Incidence and prognostic impact of FoxP3+ regulatory T cells in human gliomas. Clin Cancer Res 2008;14:5166-72.
13. Fecci PE, Ochiai H, Mitchell DA, et al. Systemic CTLA-4 blockade ameliorates glioma-induced changes to the CD4+ T cell compartment without affecting regulatory T-cell

function. Clinical cancer research : an official journal of the American Association for Cancer Research 2007;13:2158-67.
14. Grossman SA, Ye X, Lesser G, et al. Immunosuppression in patients with high-grade gliomas treated with radiation and temozolomide. Clin Cancer Res 2011;17:5473-80.
15. Lorgis V, Maura G, Coppa G, et al. Relation between bevacizumab dose intensity and high-grade glioma survival: a retrospective study in two large cohorts. Journal of neuro-oncology 2012;107:351-8.
16. de Groot JF, Fuller G, Kumar AJ, et al. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. Neuro-oncology 2010;12:233-42.
17. Keunen O, Johansson M, Oudin A, et al. Anti-VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma. Proc Natl Acad Sci U S A 2011;108:3749-54.
18. Piao Y, Liang J, Holmes L, et al. Glioblastoma resistance to anti-VEGF therapy is associated with myeloid cell infiltration, stem cell accumulation, and a mesenchymal phenotype. Neuro-oncology 2012;14:1379-92.
19. Lu KV, Chang JP, Parachoniak CA, et al. VEGF inhibits tumor cell invasion and mesenchymal transition through a MET/VEGFR2 complex. Cancer cell 2012;22:21-35.
20. DeLay M, Jahangiri A, Carbonell WS, et al. Microarray analysis verifies two distinct phenotypes of glioblastomas resistant to antiangiogenic therapy. Clinical cancer research : an official journal of the American Association for Cancer Research 2012;18:2930-42.
21. Huang Y, Goel S, Duda DG, Fukumura D, Jain RK. Vascular normalization as an emerging strategy to enhance cancer immunotherapy. Cancer Res 2013;73:2943-8.
22. Huang Y, Yuan J, Righi E, et al. Vascular normalizing doses of antiangiogenic treatment reprogram the immunosuppressive tumor microenvironment and enhance immunotherapy. Proc Natl Acad Sci U S A 2012;109:17561-6.
23. Griffioen AW. Anti-angiogenesis: making the tumor vulnerable to the immune system. Cancer immunology, immunotherapy : CII 2008;57:1553-8.
24. Johnson BF, Clay TM, Hobeika AC, Lyerly HK, Morse MA. Vascular endothelial growth factor and immunosuppression in cancer: current knowledge and potential for new therapy. Expert opinion on biological therapy 2007;7:449-60.
25. Ohm JE, Carbone DP. VEGF as a mediator of tumor-associated immunodeficiency. Immunol Res 2001;23:263-72.
26. Gabrilovich D, Ishida T, Oyama T, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo. Blood 1998;92:4150-66.
27. Gabrilovich DI, Chen HL, Girgis KR, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 1996;2:1096-103.

28. Mulligan JK, Day TA, Gillespie MB, Rosenzweig SA, Young MR. Secretion of vascular endothelial growth factor by oral squamous cell carcinoma cells skews endothelial cells to suppress T-cell functions. Human immunology 2009;70:375-82.
29. Ohm JE, Gabrilovich DI, Sempowski GD, et al. VEGF inhibits T-cell development and may contribute to tumor-induced immune suppression. Blood 2003;101:4878-86.
30. Huang X, Raskovalova T, Lokshin A, et al. Combined antiangiogenic and immune therapy of prostate cancer. Angiogenesis 2005;8:13-23.
31. Miyazaki J, Tsuzuki Y, Matsuzaki K, et al. Combination therapy with tumor-lysate pulsed dendritic cells and antiangiogenic drug TNP-470 for mouse pancreatic cancer. Int J Cancer 2005;117:499-505.
32. Shrimali RK, Yu Z, Theoret MR, Chinnasamy D, Restifo NP, Rosenberg SA. Antiangiogenic agents can increase lymphocyte infiltration into tumor and enhance the effectiveness of adoptive immunotherapy of cancer. Cancer research 2010;70:6171-80.
33. U.S. Department of Health and Human Services CfDE, and Research FaDa: FDA Project on Cancer Drug Aproval Endpoint Last updated April 20, 2007.
34. Li B, Lalani AS, Harding TC, et al. Vascular endothelial growth factor blockade reduces intratumoral regulatory T cells and enhances the efficacy of a GM-CSF-secreting cancer immunotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research 2006;12:6808-16.
35. Manning EA, Ullman JG, Leatherman JM, et al. A vascular endothelial growth factor receptor-2 inhibitor enhances antitumor immunity through an immune-based mechanism. Clinical cancer research : an official journal of the American Association for Cancer Research 2007;13:3951-9.
36. Hodi FS, Lawrence D, Lezcano C, et al. Bevacizumab plus Ipilimumab in Patients with Metastatic Melanoma. Cancer immunology research 2014;2:632-42.
37. Cohen MH, Shen YL, Keegan P, Pazdur R. FDA drug approval summary: bevacizumab (Avastin) as treatment of recurrent glioblastoma multiforme. Oncologist 2009;14:1131-8.
38. Piao Y, Liang J, Holmes L, Zurita A, al. e. Glioblastoma resistance to anti-VEGF therapy is associated with myeloid cell infiltration, stem cell accumulation, and a mesenchymal phenotype. Neuro-oncology 2012;14:1379-92.
39. Lu K, Chang J, Parachoniak C, Pandika M, Aghi M, al. e. VEGF inhibits tumor cell invasion and mesenchymal transition through a MET/VEGFR2 complex. Cancer cell 2012;22:21-35.
40. DeLay M, Jahangiri A, Carbonell WS, et al. Microarray analysis verifies two distinct phenotypes of glioblastomas resistant to antiangiogenic therapy. Clinical cancer research : an official journal of the American Association for Cancer Research 2012;18:2930-42.
41. Jain RK, Tong RT, Munn LL. Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema, and lymphatic metastasis: insights from a mathematical model. Cancer Res 2007;67:2729-35.

42. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005;307:58-62.
43. Sorensen AG, Emblem KE, Polaskova P, et al. Increased survival of glioblastoma patients who respond to antiangiogenic therapy with elevated blood perfusion. Cancer Res 2012;72:402-7.
44. Emblem KE, Mouridsen K, Bjornerud A, et al. Vessel architectural imaging identifies cancer patient responders to anti-angiogenic therapy. Nature medicine 2013;19:1178-83.
45. Huang Y, Goel S, Duda D, Fukumura D, Jain R. Vascular normalization as an emerging strategy to enhance cancer immunotherapy. Cancer research 2013;73:2943-8.
46. Lorgis V, Maura G, Coppa G, Hassani K, al. e. Relation between bevacizumab dose intensity and high-grade glioma survival: a retrospective study in two large cohorts. Journal of neuro-oncology 2012;107:351-8.
47. Taal W, Oosterkamp HM, Walenkamp AM, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. Lancet Oncol 2014;15:943-53.
48. Wick W, Brandes AA, Gorlia T, et al. EORTC 26101 phase III trial exploring the combination of bevacizumab and lomustine in patients with first progression of a glioblastoma. ASCO Meeting Abstracts 2016;34:2001.
49. Presta LG, Chen H, O'Connor SJ, et al. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 1997;57:4593-9.
50. Kim KJ, Li B, Winer J, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 1993;362:841-4.
51. Borgstrom P, Gold DP, Hillan KJ, Ferrara N. Importance of VEGF for breast cancer angiogenesis in vivo: implications from intravital microscopy of combination treatments with an anti-VEGF neutralizing monoclonal antibody and doxorubicin. Anticancer Res 1999;19:4203-14.
52. Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 2003;111:1287-95.
53. Shaheen RM, Ahmad SA, Liu W, et al. Inhibited growth of colon cancer carcinomatosis by antibodies to vascular endothelial and epidermal growth factor receptors. Br J Cancer 2001;85:584-9.
54. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335-42.
55. Giantonio BJ, Catalano PJ, Meropol NJ, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. Journal

of clinical oncology : official journal of the American Society of Clinical Oncology 2007;25:1539-44.
56. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355:2542-50.
57. Escudier B, Pluzanska A, Koralewski P, et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet 2007;370:2103-11.
58. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357:2666-76.
59. Kreisl TN, Kim L, Moore K, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2009;27:740-5.
60. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2009;27:4733-40.
61. Wong ET, Hess KR, Gleason MJ, et al. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1999;17:2572-8.
62. Levin VA, Bidaut L, Hou P, et al. Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. International journal of radiation oncology, biology, physics 2011;79:1487-95.
63. Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci U S A 2010;107:4275-80.
64. Emblem KE, Mouridsen K, Bjornerud A, et al. Vessel architectural imaging identifies cancer patient responders to anti-angiogenic therapy. Nat Med 2013.
65. Okada H, Weller M, Huang R, et al. Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. Lancet Oncol 2015;16:e534-42.
66. Glusker P, Recht L, Lane B. Reversible posterior leukoencephalopathy syndrome and bevacizumab. N Engl J Med 2006;354:980-2; discussion -2.
67. Kabbinavar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2003;21:60-5.
68. Nalluri SR, Chu D, Keresztes R, Zhu X, Wu S. Risk of venous thromboembolism with the angiogenesis inhibitor bevacizumab in cancer patients: a meta-analysis. JAMA 2008;300:2277-85.

69. Scappaticci FA, Skillings JR, Holden SN, et al. Arterial thromboembolic events in patients with metastatic carcinoma treated with chemotherapy and bevacizumab. J Natl Cancer Inst 2007;99:1232-9.
70. Hapani S, Chu D, Wu S. Risk of gastrointestinal perforation in patients with cancer treated with bevacizumab: a meta-analysis. Lancet Oncol 2009;10:559-68.
71. Chen HX, Cleck JN. Adverse effects of anticancer agents that target the VEGF pathway. Nature reviews Clinical oncology 2009;6:465-77.
72. Scappaticci FA, Fehrenbacher L, Cartwright T, et al. Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. J Surg Oncol 2005;91:173-80.
73. Miller KD, Chap LI, Holmes FA, et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2005;23:792-9.
74. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:1963-72.

15.0 APPENDICES

APPENDIX 1: KARNOFSKY AND ECOG PERFORMANCE STATUS SCALES

Status	Karnofsky	Grade	ECOG
Normal, no complaints	100	0	Fully active, able to carry on all pre-disease performance without restriction
Able to carry on normal activities. Minor signs or symptoms of disease	90	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
Normal activity with effort	80		
Care for self. Unable to carry on normal activity or to do active work	70	2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
Requires occasional assistance, but able	60		

to care for most of his needs			
Requires considerable assistance and frequent medical care	50	3	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
Disabled. Requires special care and assistance	40		
Severely disabled. Hospitalization indicated though death non-imminent	30	4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
Very sick. Hospitalization necessary. Active supportive treatment necessary	20		
Moribund	10		
Dead	0		

APPENDIX 2: IMMUNOTHERAPY RESPONSE ASSESSMENT IN NEURO-ONCOLOGY (IRANO) CRITERIA

Tumor response should be assessed every 8 weeks (+/- 1 week) for subjects treated with immunotherapy using modified RANO criteria[65] as outlined below. Clinicians may repeat response assessment more frequently as clinically indicated.

Anti-Tumor Effect Definitions

Evaluable for toxicity. All subjects who receive at least one dose of immunotherapy treatment will be evaluable for toxicity from the time of their first treatment.

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

Measurable disease. For contrast-enhancing tumors, measurable disease is defined as the bi-dimensionally, contrast-enhancing, measurable lesions with clearly defined margins by CT or MRI scan, with a minimal diameter of 1 cm, and visible on 2 slices which are at least 5 mm apart with 0 mm skip. For non-contrast-enhancing tumors, measurable disease is defined as the T2 or FLAIR lesions with a minimal diameter of 1 cm, and visible on 2 slices which are at least 5 mm apart with 0 mm skip. Measurement of tumor around a cyst or surgical cavity, if necessary, requires a minimum thickness of 3 mm. If there are too many measurable lesions to measure at each evaluation, the investigator must choose the largest two to be followed. The remaining lesions will be considered non-measureable for the purpose of objective response determination. Unless progression is observed, objective response can only be determined when all measurable and non-measurable lesions are assessed.

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

Response/Progression Categories

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

a) 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.

b) No new lesions.

c) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

d) Subjects must be on no steroids or on physiologic replacement doses only.

e) For enhancing tumors: Stable or improved non-enhancing (T2/FLAIR) lesions

f) Stable or improved clinically, for clinical signs and symptoms present at baseline and recorded to be disease related

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

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

a) Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters of all measurable 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.

b) No progression of non-measurable disease.

c) No new lesions.

d) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.

e) The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.

f) For enhancing tumors: Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.

g) Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

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

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

a) > 25% increase in sum of the products of perpendicular diameters of measurable lesions (over best response [smallest tumor size] or baseline if no decrease) on stable or increasing doses of corticosteroids

b) Any new measurable lesion that when added to the change in initial tumor(s) exceeds a 25% increase in cross-sectional area.

c) 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.

d) Failure to return for evaluation due to death or deteriorating condition

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

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

a) Does not qualify for CR, PR, or progression.

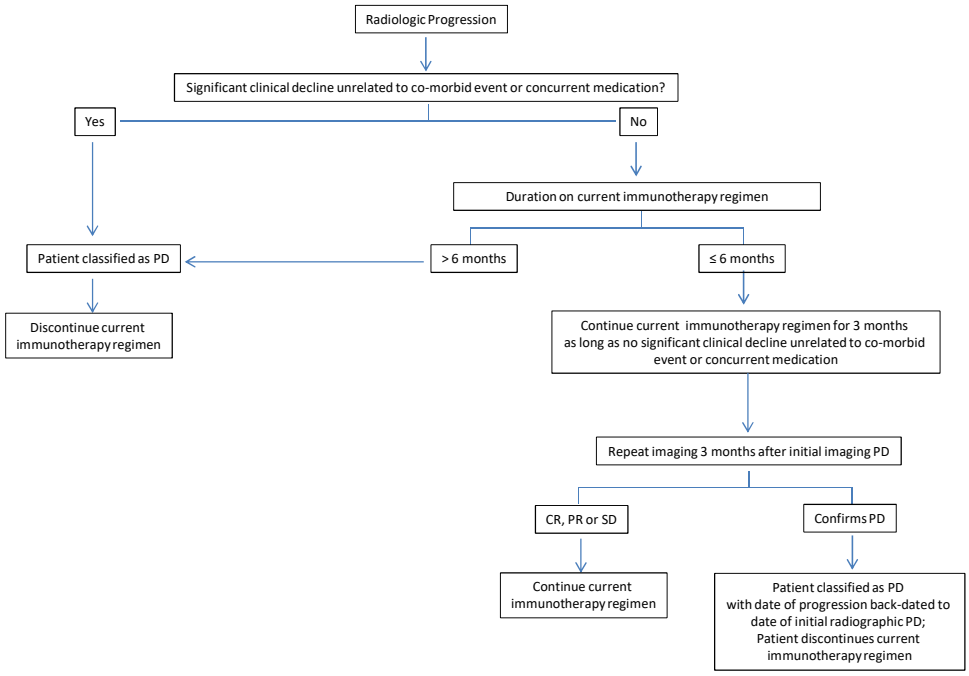
b) All measurable and non-measurable sites must be assessed using the same techniques as baseline.

c) Stable clinically.

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

Algorithm for Treatment Decision Making for Subjects with Radiographic Progression

Figure 2. iRANO algorithm for treatment decision making for radiologic progression



APPENDIX 3: SAMPLE OF DRUG ORDERING AND PHARMACY REFERENCE MATERIAL

Nivolumab (BMS-936558) Pharmacy Reference Material

- Nivolumab has a concentration of 10mg/mL and is provided in a 10mL vial. Ten or five vials are provided in a carton.

Initial Orders

- *Following submission and approval of the required regulatory documents, a supply of nivolumab may be ordered from by completing a Drug Request Form provided by BMS for this specific trial. The first request may take place upon screening of the first patient*
- *The initial order should be limited to 20 vials. Allow 5 business days for shipment of drug from BMS receipt of the Drug Request Form. Drug is protocol specific, but not patient specific. All drug product will be shipped by courier in a temperature-controlled container. It is possible that sites may have more than one nivolumab clinical study ongoing at the same time. It is imperative that only drug product designated for this protocol number be used for this study.*
- *Pharmacy supplies not provided by BMS: Empty IV bags/containers, approved diluents, In-line filters and infusion tubing*

Re-Supply

- *Drug re-supply request form should be submitted electronically business days before the expected delivery date. Deliveries will be made Tuesday through Friday.*
- *When assessing need for resupply, institutions should keep in mind the number of vials used per treatment dose, and that shipments may take 14 business days from receipt of request. Drug is not patient-specific. Be sure to check with your pharmacy regarding existing investigational stock to assure optimal use of drug on hand.*

Drug Excursions

- *Drug excursions should be reported immediately to BMS on the form provided with the study-specific drug order form*

Please refer to the most recent version of the Investigator Brochure for additional information.

Storage Conditions & Handling:

- Store at 2-8°C (36-46°F), protect from light, freezing, and shaking.
- If any temperature excursions are encountered during storage, please report these to BMS for assessment via the Temperature Excursion Response Form.
- As with all injectable drugs, care should be taken when handling and preparing nivolumab. Whenever possible, nivolumab should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents applying aseptic technique.
- Partially used vials should be disposed at the site following procedures for the disposal of anticancer drugs.

After final drug reconciliation, unused nivolumab vials should be disposed at the site following procedures for the disposal of anticancer drugs. For further information, please either discuss with your BMS CSR&O protocol manager or refer to your site IP Destruction policies and procedures

Use Time/Stability: Please refer to section 3.2.3 of the current Investigator Brochure. Due to parameters surrounding the use time of Nivolumab, the time of preparation should be noted in the Pharmacy Source documents [accountability logs] or in study files as required for investigator sponsored research [FDA and GCP]

The administration of BMS-936558-01 injection prepared for dosing nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 20 hours in a refrigerator at under refrigeration conditions (2°-°-8°C (, 36°-46°F) and used within 4for up to 24 hours, and a maximum of 4 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and under room light. The maximum 4-hour period under room temperature and room light conditions for undiluted and diluted solutions of BMS-936558-01 injection in the IV bag should be inclusive of the includes the product administration period.

Preparation and Administration:

6. Visually inspect the drug product solution for particulate matter and discoloration prior to administration. Discard if solution is cloudy, if there is pronounced discoloration (solution may have a pale-yellow color), or if there is foreign paticulate matter other than a few translucent-to-white, amorphous particles.

   *Note: Mix by **gently** inverting several times. **Do not** shake.*

7. Aseptically withdraw the required volume of nivolumab solution into a syringe, and dispense into an IV. bag. If multiple vials are needed for a subject, it is important to use a separate sterile syringe and needle for each vial to prevent problems such as dulling of needle tip, stopper coring, repeated friction of plunger against syringe barrel wall. **Do not** enter into each vial more than once. **Do not** administer study drug as an IV push or bolus injection

8. Add the appropriate volume of 0.9% Sodium Chloride Injection solution or 5% Dextrose Injection solution. *It is acceptable to add nivolumab solution from the vials into an appropriate pre-filled bag of diluent.*

   Note: Nivolumab infusion concentration must be at or above the minimum allowable concentration of 0.35 mg/mL [IBV13 Addendum Section 3.2.2]

   Note: It is not recommended that so-called "channel" or tube systems are used to transport prepared infusions of nivolumab.

9. Attach the IV bag containing the nivolumab solution to the infusion set and filter.

10. At the end of the infusion period, flush the line with a sufficient quantity of approved diluents.

APPENDIX 4: ADVERSE EVENT REPORTING

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to BMS Worldwide Safety.
- If the BMS safety address is not included in the protocol document (e.g. multicenter studies where events are reported centrally), the procedure for safety reporting must be reviewed/approved by the BMS Protocol Manager. Procedures for such reporting must be reviewed and approved by BMS prior to study activation.
- The BMS SAE form should be used to report SAEs. If the BMS form cannot be used, another acceptable form (i.e. CIOMS or Medwatch) must be reviewed and approved by BMS. The BMS protocol ID number must be included on whatever form is submitted by the Sponsor/Investigator.
- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring after these time periods that is believed to be related to study drug or protocol-specified procedure.
-
- In accordance with local regulations, BMS will notify investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (i.e., not previously described in the IB). In the European Union (EU), an event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Investigator notification of these events will be in the form of an expedited safety report (ESR).
    - Other important findings which may be reported by the as an ESR include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (e.g., animal) study, important safety recommendations from a study data monitoring committee, or sponsor decision to end or temporarily halt a clinical study for safety reasons.

- Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.
- In addition, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

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 Pregnancy Surveillance Form.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

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

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

All SAEs should be followed to resolution or stabilization.

For studies conducted under an Investigator IND in the US include the following:

For studies conducted under an Investigator IND in the US, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all

other SAEs) after the investigator's or institution's initial receipt of the information. BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.

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

All SAEs should simultaneously be faxed or e-mailed to BMS at:

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

- An SAE report should be completed for any event where doubt exists regarding its seriousness.
- For studies with long-term follow-up periods in which safety data are being reported, include the timing of SAE collection in the protocol.
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.
- If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)
- If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization. All SAEs should be followed to resolution or stabilization.

DEFINITIONS

The protocol must include a definition for Serious Adverse Events (SAE)

SERIOUS ADVERSE EVENTS

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

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

NOTE: (PI- determines if this information should be included. This is provided as supplemental information that is included in BMS-sponsored trials)

The following hospitalizations are not considered SAEs in BMS clinical studies:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases

- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

Potential Drug Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs Potential drug induced liver injury is defined as:

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

ADVERSE EVENTS

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

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

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

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

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

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

NONSERIOUS ADVERSE EVENT

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

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

Nonserious Adverse Event Collection and Reporting

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.

Laboratory Test Abnormalities

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

The following laboratory abnormalities should be documented and reported appropriately:

Any laboratory test result that is clinically significant or meets the definition of an SAE

Any laboratory abnormality that required the subject to have study drug discontinued or interrupted

Any laboratory abnormality that required the subject to receive specific corrective therapy.

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety).

The investigator must immediately notify Worldwide Safety @BMS of this event via the Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form [provided upon request from BMS]

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

Overdose

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

Other Safety Considerations

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

APPENDIX 5 Recommended MRI Sequences

	3D T1w Pre	Ax 2D FLAIR	Ax 2D DWI	Contrast Injection [a]	SE EPI Perf	Ax 2D T2w	3D T1w Post[b]
Sequence	IR-GRE[d,e]	TSE[c]	EPI[f]		EPI	TSE[c]	IR-GRE[d,e]
Plane	Sagittal/Axial	Axial	Axial		Axial	Axial	Axial/Sagittal
Mode	3D	2D	2D		2D	2D	3D
TR [ms]	2100[g]	>6000	>5000		2260	>2500	2100[g]
TE [ms]	Min	100-140	Min		78	80-120	Min
TI [ms]	1100[h]	2000 - 2500					1100[h]
Flip Angle	10º-15º	90º/≥160º	90º/180º			90º/≥160º	10º-15º
Frequency	256	≥256	128		128	≥256	256
Phase	256	≥256	128		128	≥256	256
NEX	≥1	≥1	≥1		1	≥1	≥1
FOV	256mm	240mm	240mm		280	240mm	256mm
Slice Thickness	1mm	3mm	3mm		10mm	3mm	1mm
Gap/Spacing	0	0	0		0	0	0
Diffusion Options			b = 0, 500, and 1000 s/mm^2 ≥3 directions				
Parallel Imaging	Up to 2x	Up to 2x	Up to 2x			Up to 2x	Up to 2x
Scan Time (Approx)	4:53	3:39	3:20		1:36	2:17	4:53

A: Recommended 3T Protocol[a] 0.1 mmol/kg or up to 20cc (single, full dose) of MR contrast.

[b] Post-contrast 3D axial T1-weighted images should be collected with identical parameters to pre-contrast 3D axial T1-weighted images

[c] TSE = turbo spin echo (Siemens & Philips) is equivalent to FSE (fast spin echo; GE, Hitachi, Toshiba)

[d] IR-GRE = inversion-recovery gradient-recalled echo sequence is equivalent to MPRAGE = magnetization prepared rapid gradient-echo (Siemens & Hitachi) and the inversion recovery spoiled gradient-echo (IR-SPGR or Fast SPGR with inversion activated or BRAVO; GE), 3D turbo field echo (TFE; Philips), or 3D fast field echo (3D Fast FE; Toshiba).

[e] A 3D acquisition without inversion preparation will result in different contrast compared with MPRAGE or another IR-prepped 3D T1-weighted sequences and therefore should be avoided.

[f] In the event of significant patient motion, a radial acquisition scheme may be used (e.g. BLADE [Siemens], PROPELLER [GE], MultiVane [Philips], RADAR [Hitachi], or JET [Toshiba]); however, this acquisition scheme is can cause significant differences in ADC quantification and therefore should be used only if EPI is not an option.

[g] For Siemens and Hitachi scanners. GE, Philips, and Toshiba scanners should use a TR = 5-15ms for similar contrast.

[h] For Siemens and Hitachi scanners. GE, Philips, and Toshiba scanners should use a TI = 400-450ms for similar contrast.

Acronyms:
Ax = Axial; ADC = apparent diffusion coefficient. FLAIR = fluid attenuated inversion recovery; DWI = diffusion-weighted imaging; 3D = three dimensional; TSE = turbo spin echo; EPI = echo planar imaging; MPRAGE = magnetization prepared rapid gradient-echo; A/P = anterior to posterior; R/L = right to left; NEX = number of excitations or averages; FOV = field of view; IR-GRE = inversion-recovery gradient-recalled echo

B: Recommended 1.5T Protocol

	3D T1w Pre	Ax 2D FLAIR	Ax 2D DWI	Contrast Injection [a]	Ax 2D T2w	3D T1w Post[b]
Sequence	IR-GRE[d,e]	TSE[c]	EPI[f]		TSE[c]	IR-GRE[d,e]
Plane	Sagittal/Axial	Axial	Axial		Axial	Sagittal/Axial
Mode	3D	2D	2D		2D	3D
TR [ms]	2100[g]	>6000	>5000		>3500	2100[g]
TE [ms]	Min	100-140	Min		100-120	Min
TI [ms]	1100[h]	2000-2500				1100[h]
Flip Angle	10º-15º	90º/≥160º	90º/180º		90º/≥160º	10º-15º
Frequency	≥172	≥256	128		≥256	≥172
Phase	≥172	≥256	128		≥256	≥172
NEX	≥1	≥1	≥1		≥1	≥1
FOV	256mm	240mm	240mm		240mm	256mm
Slice Thickness	≤1.5mm	≤4mm	≤4mm		≤4mm	≤1.5mm
Gap/Spacing	0	0	0		0	0
Diffusion Options[i]			*b* = 0, 500, and 1000 s/mm^2 ≥3 directions			
Parallel Imaging	No	Up to 2x	Up to 2x		Up to 2x	No
Scan Time (Approx)	5-10 min	4-5 min	3-5 min		3-5 min	5-10 min

[a] 0.1 mmol/kg or up to 20cc (single, full dose) of MR contrast.
[b] Post-contrast 2D axial T1-weighted images should be collected with identical parameters to pre-contrast 2D axial T1-weighted images
[c] TSE = turbo spin echo (Siemens & Philips) is equivalent to FSE (fast spin echo; GE, Hitachi, Toshiba)
[d] IR-GRE = inversion-recovery gradient-recalled echo sequence is equivalent to MPRAGE = magnetization prepared rapid gradient-echo (Siemens & Hitachi) and the inversion recovery spoiled gradient-echo (IR-SPGR or Fast SPGR with inversion activated or BRAVO; GE), 3D turbo field echo (TFE; Philips), or 3D fast field echo (3D Fast FE; Toshiba).
[e] A 3D acquisition without inversion preparation will result in different contrast compared with MPRAGE or another IR-prepped 3D T1-weighted sequences and therefore should be avoided.
[f] In the event of significant patient motion, a radial acquisition scheme may be used (e.g. BLADE [Siemens], PROPELLER [GE], MultiVane [Philips], RADAR [Hitachi], or JET [Toshiba]); however, this acquisition scheme is can cause significant differences in ADC quantification and therefore should be used only if EPI is not an option.
[g] For Siemens and Hitachi scanners. GE, Philips, and Toshiba scanners should use a TR = 5-15ms for similar contrast.
[h] For Siemens and Hitachi scanners. GE, Philips, and Toshiba scanners should use a TI = 400-450ms for similar contrast.
[i] Older model MR scanners that are not capable of >2 *b*-values should use *b* = 0 and 1000 s/mm^2.

Acronyms:
Ax = Axial; ADC = apparent diffusion coefficient. FLAIR = fluid attenuated inversion recovery; DWI = diffusion-weighted imaging; 3D = three dimensional; TSE = turbo spin echo; EPI = echo planar imaging; MPRAGE = magnetization prepared rapid gradient-echo; A/P = anterior to posterior; R/L = right to left;

NEX = number of excitations or averages; FOV = field of view; IR-GRE = inversion-recovery gradient-recalled echo

Appendix 6 Hepatoxicity Article

International Journal of Molecular Sciences

Review

Hepatotoxicity by Drugs: The Most CommonImplicated Agents

Einar S. Björnsson

Department of Internal Medicine, Division of Gastroenterology and Hepatology, The National University Hospital of Iceland and The Faculty of Medicine, The University of Iceland, 108 Reykjavik, Iceland; einarsb@landspitali.is; Tel.: +354-543-6180; Fax: +354-543-4834

Academic Editors: Rolf Teschke and Raúl J. Andrade

Received: 4 January 2016; Accepted: 1 February 2016; Published: 6 February 2016

Abstract: Idiosyncratic drug-induced liver injury (DILI) is an underreported and underestimated adverse drug reaction. Information on the documented hepatotoxicity of drugs has recently been made available by a website that can be accessed in the public domain: LiverTox (http://livertox.nlm.nih.gov). According to critical analysis of the hepatotoxicity of drugs in LiverTox, 53% of drugs had at least one case report of convincing reports of liver injury. Only 48 drugs had more than 50 case reports of DILI. Amoxicillin-clavulanate is the most commonly implicated agent leading to DILI in the prospective series. In a recent prospective study, liver injury due to amoxicillin-clavulanate was found to occur in approximately one out of 2300 users. Drugs with the highest risk of DILI in this study were azathioprine and infliximab.

Keywords: hepatotoxicity; drugs; drug-induced liver injury; idiosyncratic

1. Introduction

Drug-induced liver injury (DILI) is a frequent differential diagnosis in patients with acute liver injury without obvious etiology. Apart from exclusion of competing etiologies, an important element in the diagnostic process is the information about the known and potential hepatotoxicity of the agent.

However, data on hepatotoxicity is not always easily accessible. All drugs approved by regulatory authorities are accompanied by package inserts, called the "patient information" leaflet in Europe and "prescribing information" in the United States [1,2]. Adverse liver reactions are often mentioned in these product labels (package inserts) as a part of the prescribing information. However, it is not always clear whether this is related to enzyme elevations in clinical trials and/or clinically apparent liver injury. Thus, from package inserts of prescribed medications the clinician can get the idea that adverse drug reactions are side effects of most drugs. It has recently been demonstrated that this information is insufficient and even misleading [3]. There was also a substantial discrepancy in the official package inserts and liver disease labeling between Europe and the United States [3]. The documentation of the hepatotoxicity of drugs in the medical literature is very variable.

Some drugs have been convincingly documented to cause liver injury in numerous case reports and case series. Many such drugs have a known clinical signature (phenotype) of liver injury and causality has been further documented by instances of a positive rechallenge [4,5]. Examples are chlorpromazine, halothane, isoniazid and amoxicillin-clavulanate. In early DILI research, halothane and chlorpromazine were commonly reported causes of hepatotoxicity [6]. However, with some drugs, although marketed for many decades, only a single case report or very few reports of liver injury have been published. Case reports are often not well described and critical clinical information is frequently lacking [7]. A recent study found that reports of drug-induced liver diseases often did not provide the data needed to determine the causes of suspected adverse effects [7]. Although a case report has been published, it does not prove that the drug is hepatotoxic.

Int. J. Mol. Sci. 2016, 17, 224; doi:10.3390/ijms17020224
www.mdpi.com/journal/ijms

A newly established website, LiverTox® [8], was an attempt to provide up-to-date, accurate, and easily accessible information on the diagnosis, causes, frequency and patterns of liver injury attributable to both prescription and nonprescription medications. In LiverTox® there is data on almost all medications marketed in the United States, both on those who have been reported to cause liver

injury and those without reports of liver injury. Although in LiverTox® a thorough literature search has been undertaken and is provided, no attempt has been made to judge the quality of the published reports or the causality of the suspected liver injury reported.

In a recently published paper, drugs in LiverTox® were classified into categories, using all reports in this website [9]. For drugs with rather few reports (<12), the Rousel Uclaf Causality Assessment Method (RUCAM) was used [10]. In this critical analysis, many of the published reports did not stand up to critical review and currently there is no convincing evidence for some drugs with reported hepatotoxicity to be hepatotoxic [9]. Although certain drugs have a distinct phenotype such as isoniazid, which generally leads to a hepatocellular pattern or chlorpromazine cholestatic liver damage, many drugs can lead to both hepatocellular and cholestatic injury. Listing all types of patterns that have been reported for all these drugs is unfortunately not possible in this paper.

2. Categories of Hepatotoxicity

In the creation of LiverTox, drugs were arbitrarily divided into four different categories of likelihood for causing liver injury based on reports in the published literature [8]. Category A with >50 published reports, B with >12 but less than 50, C with >4 but less than 12, and D with one to three cases. In the Hepatology paper, drugs were categorized based on these numbers and another category, T, was added for agents leading to hepatotoxicity mainly in higher-than-therapeutic doses [9]. The number of published cases was counted unless >100 cases were found. The analysis was based mainly on published case reports, but case series were used if a formal causality assessment had been undertaken. In the analysis of the hepatotoxicity of drugs found in LiverTox, fewer drugs than expected had documented hepatotoxicity. Among 671 drugs available for analysis, 353 (53%) had published convincing case reports of hepatotoxicity. Thus, overall, 47% of the drugs listed in LiverTox did not have evidence of hepatotoxicity. This is at odds with product labeling which very frequently lists liver injury as adverse reaction to drugs [3]. It has to be taken into consideration that 116/863 (13%) of marketed agents had be excluded from the analysis. New drugs approved within the last five years were not included as most instances of hepatotoxicity appear in the post-marketing phase [11]. Metals

(iron, nickel, arsenic), illegal substances (cocaine, opium, heroin), and infrequently used and/or not available (not marketed currently) drugs were also excluded [9]. Herbal and dietary supplements listed in LiverTox were not included in the category analysis.

Among the 671 drugs available for analysis, the proportions of the drugs in the different categories were: A, 48 (14%); B, 76 (22%); C, 96 (27%); and D, 126 (36%). A total of 318 (47%) drugs have not been implicated (category E).

In general, drugs in categories A and B were more likely than those in C and D to have been marketed for a long time, and both were more likely to have at least one fatal case of liver injury and reported cases of positive rechallenge. There is little doubt that drugs with >50 or 100 published reports of DILI such as category A drugs are hepatotoxic. The same is probably true for the vast majority of drugs in category B. However, in categories C and D with one to 12 cases reported, it is still not clear whether these agents are really hepatoxic drugs.

3. Category A

Although drugs in this category (n = 48) were supposed to have >50 case reports of liver injury associated with the use of these drugs, 81% of the drugs had >100 cases reported. Interestingly, overall, 92% of these drugs had documented positive rechallenge. In Table 1, the category A drugs are illustrated with the indication and/or class of drug. These agents in category A are the real potential

hepatotoxins and clinicians should be aware of that when evaluating the risk-benefit ratio of drug therapy. Treatment with these drugs should motivate physicians to guide patients about potential symptoms of liver injury when taking these drugs and about prompt discontinuation if these symptoms occur. All except one entity (estrogens-progestins) or 98% had at least one convincing case that was associated with fatal outcome. All of these drugs except telithromycin had been approved for marketing for more than 15 years and 63% for more than 35 years [9]. The most common types of drugs were antimicrobials among 33% of the drugs, followed by drugs acting on the central nervous system (12.5%), cardiovascular (12.5%), rheumatologic (12.5%), antineoplastic (10%), endocrine (6%) and other types of drugs (13%). Although antimicrobials were the most common agents among drugs, antimicrobials were also the most common agents in categories B (30%), C (19%)

and D (27%). Antibiotics have been shown to be the dominating type of drug in both prospective and retrospective studies on DILI [12–16]. There is unfortunately not enough room to discuss many of these well-documented hepatotoxic agents. As mentioned in the abstract, azathioprine and infliximab have in one study been found to be associated with the highest risk of liver injury [9]. Both hepatocellular and cholestatic injury has been described due to azathioprine [8,9]. Despite the common problem of hepatotoxicity with azathioprine, there is a lack of studies with a significant number of well-characterized patients with this type of liver injury.

Table 1. Drugs that, according to analysis of data in LiverTox [8], have been associated with more than 100 cases of drug-induced liver injury.

Drug Drug	Class/Indication
1. Allopurinol	Gout prophylaxis
2. Amiodarone	Arrhythmia
3. Amoxicillin-clavulanate	Antibiotic
4. Anabolic steroids	Body building
5. Atorvastatin	Lipid lowering agent
6. Azathioprine/6-Mercaptopurine	Immunosuppressive agent
7. Busulfan	Malignancy
8. Carbamazepine	Antiepileptic
9. Chlorpromazine	Psychosis
10. Contraceptives	Birth control
11. Dantrolene	Muscle relaxant
12. Diclofenac	NSAID
13. Didanosine	Antimicrobial
14. Disulfiram	Substance abuse agent
15. Efavirenz	Antimicrobial

16. Erythromycin	**Antimicrobial**
17. Floxuridine	**Antineoplastic**
18. Flucloxacillin	**Antimicrobial**
19. Flutamide	**Antineoplastic**
20. Gold salts	**Immunosuppressive agent**
21. Halothane	**Anaesthetic**
22. Hydralazine	**Antihypertensive**
23. Ibuprofen	**NSAID**
24. Infliximab	**Immunosuppressive agent**
25. Interferon alpha/Peginterferon	**Antimicrobial**
26. Interferon beta	**Multiple Sclerosis**
27. Isoniazid	**Antituberculosis**
28. Ketoconazole	**Antifungal**
29. Methotrexate	**Immunosuppressive agent**
30. Methyldopa	**Antihypertensive**
31. Minocycline	**Antibiotic**
32. Nevirapine	**Antimicrobial**
33. Nimesulide	**NSAID**
34. Nitrofurantoin	**Antibiotic**

Int. J. Mol. Sci. 2016, 17, 224 4 of 7

Table 1. Cont.

Drug Drug	**Class/Indication**
35. Phenytoin	**Antiepileptic**

36. Propylthiouracil	**Antithyroid**
37. Quinidine	**Arrhythmia**
38. Pyrazinamide	**Antituberculosis**
39. Rifampin	**Antituberculosis**
40. Simvastatin	**Lipid lowering agent**
41. Sulfamethoxazole/Trimethoprim	**Antibiotic**
42. Sulfazalazine	**Antibiotic**
43. Sulfonamides	**Antibiotic**
44. Sulindac	**NSAID**
45. Telithromycin	**Antibiotic**
46. Thioguanine	**Antineoplastic**
47. Ticlopidine	**Platelet inhibitor**
48. Valproate	**Antiepilepitic**

4. Category B

As mentioned above, most of these drugs with >12 and up to 50 case reports of DILI published probably carry hepatotoxic potential. This seems particularly true for drugs with reports of documented rechallenge, which had been reported in at least one case in 38% of the drugs [9]. In comparison with category A drugs, which almost exclusively had been associated with fatality, approximately 50% of category B drugs had been associated with a fatal outcome. Thus, in drugs with less frequent reporting of liver injury in category B, only 38% had rechallenge reported vs. 92% in category A, which suggests that the "proof" of hepatotoxicity is not there for all these drugs. In category B, 13/76 (17%) drugs with >30 cases reported are shown in Table 2.

Table 2. Drugs in category B (>12 and >40 cases) that, according to analysis of data in LiverTox [8],have been associated with >30 published case reports of drug induced liver injury.

Drug	Drug Class/Indication
Amodiaquine	Antimicrobial
Azithromycin	Antimicrobial
Chlorzoxazone	Muscle relaxant
Cyproterone	Antineoplastic
Heparin	Anticoagulant
Imatinib	Antineoplastic
Irinotecan	Antineoplastic
Levofloxacin/Ofloxacin	Antimicrobial
Oxacillin	Antimicrobial
Phenobarbital	Antiepileptic
Stavudine	Antimicrobial
Tamoxifen	Antineoplastic
Terbinafine	HIV

5. Categories C, D and E

Overall, 222/353 (63%) of drugs in LiverTox® with hepatotoxicity fall into categories C and D. Compared with category D, with only one to three cases reported, category C (<12 and >4 case reports) drugs were more likely to have rechallenge reports, with 26% vs. 11%, and fatal cases of 23% and 7%, respectively. A positive rechallenge is usually defined with biochemical criteria, showing recurrence of liver test abnormalities upon readministration of the drug, due to either intentional or inadvertent re-exposure [4,5]. This is generally considered to be the gold standard of the diagnosis of drug-induced liver injury. A documented positive rechallenge provides more evidence of the hepatotoxicity of a

Int. J. Mol. Sci. 2016, 17, 224 5 of 7

given drug. Given the frequency of case reports with drugs in categories A and B, there seems little doubt that drugs in these categories can lead to hepatotoxicity and little need to do a strict causality assessment of reports with these drugs.

However, in category C, consisting of 4–11 case reports, the hepatotoxicity of some drugs can be put into question. To illustrate this, 16 drugs in this category only had case reports with a possible likelihood score according to RUCAM. None of these drugs had documented fatal liver reactions or rechallenge. Thus, it can be concluded that these drugs do not have a well-documented hepatotoxicity, although liver injury with their use cannot be excluded. The poorly documented exclusion of competing causes, as well as the use of other concomitant drugs, made a causality assessment difficult. This has been problematic in many reports of suspected hepatotoxicity with human immunodeficiency virus (HIV) drugs [17–19]. It is very important that observations of hepatotoxicity of new drugs should lead to well-documented case reports with detailed clinical and biochemical information.

The analysis reported in the Hepatology paper revealed that many drugs labeled as hepatotoxic and with a single or few case reports suggesting hepatotoxicity did not fulfill causality criteria by use of the RUCAM instrument [9].

6. Common Drugs Leading to Liver Injury in Drug-Induced Liver Injury (DILI) Studies

As mentioned above, antibiotics have, in all prospective studies, been found to be the most common drugs leading to hepatotoxicity [12–16]. In the most recently published series from the DILIN cohort in the US, antimicrobials, including antibacterial agents and antituberculosis agents, were approximately 46% of all DILI cases [20]. Furthermore, among the top 10 drugs in the DILIN registry, all drugs except one (Diclofenac) are antibiotics [20]. Table 3 illustrates the five most common drugs associated with liver injury in at least three prospective studies. Interestingly, all of these drugs belong to category A.

Table 3. The top five implicated drugs in three prospective studies on DILI, in Spain (Andrade et al. [12] 2005), liver injury in drug-induced liver Injury (DILI) study from the US (Chalasani et al. [13] 2013) and a prospective study from Iceland (Bjornsson et al. [14] 2015).

Spanish Registry	DILIN Study	Icelandic Study
Amoxicillin-clavulanate	Amoxicillin-clavulanate	Amoxicillin-clavulanate

Isoniazid	**Isoniazid**	**Diclofenac**
RIP + INH + PIZ	**Nitrofurantoin**	**Azathioprine**
Flutamide	**SMZ/TMP**	**Infliximab**
Ibuprofen	**Minocycline**	**Nitrofurantoin**

RIP + INH + PIZ: Rifampin, Isoniazid and Pyrazinamide; SMZ/TMPSulfamethoxazole/Trimethoprim.

In India, anti-tuberculous drugs (58%), anti-epileptics (11%), olanzapine (5%), and dapsone (5%) were the most common causes [16]. A unified list of drugs associated with DILI was recently established [21]. Overall 385 individual drugs were identified; 319 drugs were identified in three DILI registries, i.e., from Spain, Sweden and the US. The 10 most frequently implicated drugs were: amoxicillin-clavulanate, flucloxacillin, erythromycin, diclofenac, sulfamethoxazole/Trimethoprim, isoniazid, disulfiram, Ibuprofen and flutamide [12–14,21].

7. Risk of DILI among Patients Using Potentially Hepatotoxic Drugs

Previously, data on numbers needed to harm drug users in terms of liver injury has been limited. Several retrospective case control cohort studies using the General Practitioners Research Database (GPRD) were the first studies on this [22–24].

A risk of DILI greater than 100 per 100,000 users was found for chlorpromazine and isoniazid. Drugs with an intermediate risk were amoxicillin-clavulanic acid and cimetidine, with a risk of one per 10 per 100,000 users [24]. All other drugs were found to be less than 10 per 100,000 users.

Int. J. Mol. Sci. 2016, 17, 224 6 of 7

The following drugs were most strongly associated with DILI: Chlorpromazine, amoxicillin-clavulanic acid, flucloxacillin, macrolides, tetracyclines, metoclopramide, chlorpheniramine, betahistine, sulfasalazine, azathioprine, diclofenac, and antiepileptics The highest crude incidence rates were one per 739 users (chlorpromazine), one per 1103 (azathioprine), one per 1000 (sulfasalazine), and one per 11,688 (amoxicillin-clavulanate). The limitations of this study were the retrospective design with a lack of complete data regarding diagnostic testing and a lack of data on over-the-counter drugs and herbal agents [24]. In a recent

prospective study on DILI from Iceland, data on the use of drugs was available [9]. The risk of DILI among patients using potentially hepatotoxic drugs could therefore be calculated. Amoxicillin-clavulanate-induced liver injury was found in one of 2350 outpatient users, which was higher among those who were hospitalized already, one of 729. This might be due to a detection bias, with more routine testing of the liver in the hospital, but it cannot be excluded that sicker patients are more susceptible to liver injury from this drug. The incidence rates were higher than previously reported, with the highest being one of 133 users for azathioprine and one of 148 for infliximab.

Acknowledgments: No specific grants were obtained for research work presented in this paper and no funds for publishing in open access.

Conflicts of Interest: The author declares no conflict of interest.

References

1. European Medicines Agency. Available online: http://www.ema.europa.eu (accessed on 1 May 2015).

2. Physician Desk Reference (PDR). Available online: http://www.pdr.net (accessed on 1 May 2015).

3. Bjornsson, E.S.; Jacobsen, E.I.; Einarsdottir, R.; Chalasani, N. Discrepancies in liver disease labeling in the package inserts of commonly prescribed medications. Gastroenterology 2015, 148, 269–273. [CrossRef]

[PubMed]

4. Andrade, R.J.; Robles, M.; Lucena, M.I. Rechallenge in drug-induced liver injury: The attractive hazard.

Expert Opin. Drug Saf. 2009, 8, 709–714. [CrossRef] [PubMed]

5. Papay, J.I.; Clines, D.; Rafi, R.; Yuen, N.; Britt, S.D.; Walsh, J.S.; Hunt, C.M. Drug-induced liver injury following positive drug rechallenge. Regul. Toxicol. Pharmacol. 2009, 54, 84–90. [CrossRef] [PubMed]

6. Zimmerman, H.J. Drug-induced liver disease. In Diseases of the Liver, 8th ed.; Sciff, E.R., Sorrell, M.F.,

Maddrey, W.C., Eds.; Schiff's Lippincott-Raven Publishers: Philadelphia, PA, USA, 1999; pp. 973–1064.

7. Agarwal, V.K.; McHutchison, J.G.; Hoofnagle, J.H. Important elements for the diagnosis of drug-induced liver injury. Clin. Gastroenterol. Hepatol. 2010, 8, 463–470. [CrossRef] [PubMed]

8. LiverTox. Available online: http://livertox.nlm.nih.gov (accessed on 1 May 2015).

9. Björnsson, E.S.; Hoofnagle, J.H. Categorization of drugs implicated in causing liver injury: Critical assessment based upon published case reports. Hepatology 2015. [CrossRef] [PubMed]

10. Danan, G.; Teschke, R. RUCAM in drug and herb induced liver injury: The update. Int. J. Mol. Sci. 2016, 17. [CrossRef] [PubMed]

11. Senior, J.R. Evolution of the Food and Drug Administration approach to liver safety assessment for new drugs: Current status and challenges. Drug Saf. 2014, 37, S9–S17. [CrossRef] [PubMed]

12. Andrade, R.J.; Lucena, M.I.; Fernandez, M.C.; Pelaez, G.; Pachkoria, K.; García-Ruiz, E.; García-Muñoz, B.; González-Grande, R.; Pizarro, A.; Durán, J.A.; et al. Drug-induced liver injury: An analysis of 461 incidences submitted to the Spanish registry over a 10-year period. Gastroenterology 2005, 129, 512–521. [CrossRef]

[PubMed]

13. Chalasani, N.; Fontana, R.J.; Bonkovsky, H.L.; Watkins, P.B.; Davern, T.; Serrano, J.; Yang, H.; Rochon, J. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. Gastroenterology 2008, 135, 1924–1934. [CrossRef] [PubMed]

14. Bjornsson, E.S.; Bergmann, O.M.; Bjornsson, H.K.; Kvaran, R.B.; Olafsson, S. Incidence, presentation and outcomes in patients with drug-induced liver injury in the general population of Iceland. Gastroenterology 2013, 144, 1419–1425. [CrossRef] [PubMed]

15. Björnsson, E.; Olsson, R. Outcome and prognostic markers in severe drug-induced liver disease. Hepatology 2005, 42, 481–489. [CrossRef] [PubMed]

16. Devarbhavi, H.; Dierkhising, R.; Kremers, W.K.; Sandeep, M.S.; Karanth, D.; Adarsh, C.K. Single-center experience with drug-induced liver injury from India: Causes, outcome, prognosis, and predictors of mortality. Am. J. Gastroenterol. 2010, 105, 2396–2404. [CrossRef] [PubMed]

17. Bräu, N.; Leaf, H.L.;Wieczorek, R.L.; Margolis, D.M. Severe hepatitis in three AIDS patients treated with indinavir. Lancet 1997, 349, 924–925. [CrossRef]

18. Vergis, E.; Paterson, D.L.; Singh, N. Indinavir-associated hepatitis in patients with advanced HIV infection. Int. J. STD AIDS 1998, 9, 53. [CrossRef] [PubMed]

19. Pavel, S.; Burty, C.; Alcaraz, I.; de la Tribonnière, X.; Baclet, V.; Ajana, F.; Mouton, Y. Severe liver toxicity in postexposure prophylaxis for HIV infection with a zidovudine, lamivudine and fosamprenavir/ritonavir regimen. AIDS 2007, 21, 268–269. [CrossRef] [PubMed]

20. Chalasani, N.; Bonkovsky, H.L.; Fontana, R.; Lee,W.; Stolz, A.; Talwalkar, J. Features and outcomes of 889 patients with drug-induced liver injury: The DILIN prospective study. Gastroenterology 2015, 148, 1340–1352. [CrossRef] [PubMed]

21. Suzuki, A.; Andrade, R.J.; Bjornsson, E.; Lucena, M.I.; Lee, W.M.; Yuen, N.A.; Hunt, C.M.; Freston, J.W. Unified list of drugs causing hepatotoxicity and the reporting frequency of liver events in the WHO vigibaset: International collaborative work. Drug Saf. 2010, 33, 503–522. [CrossRef] [PubMed]

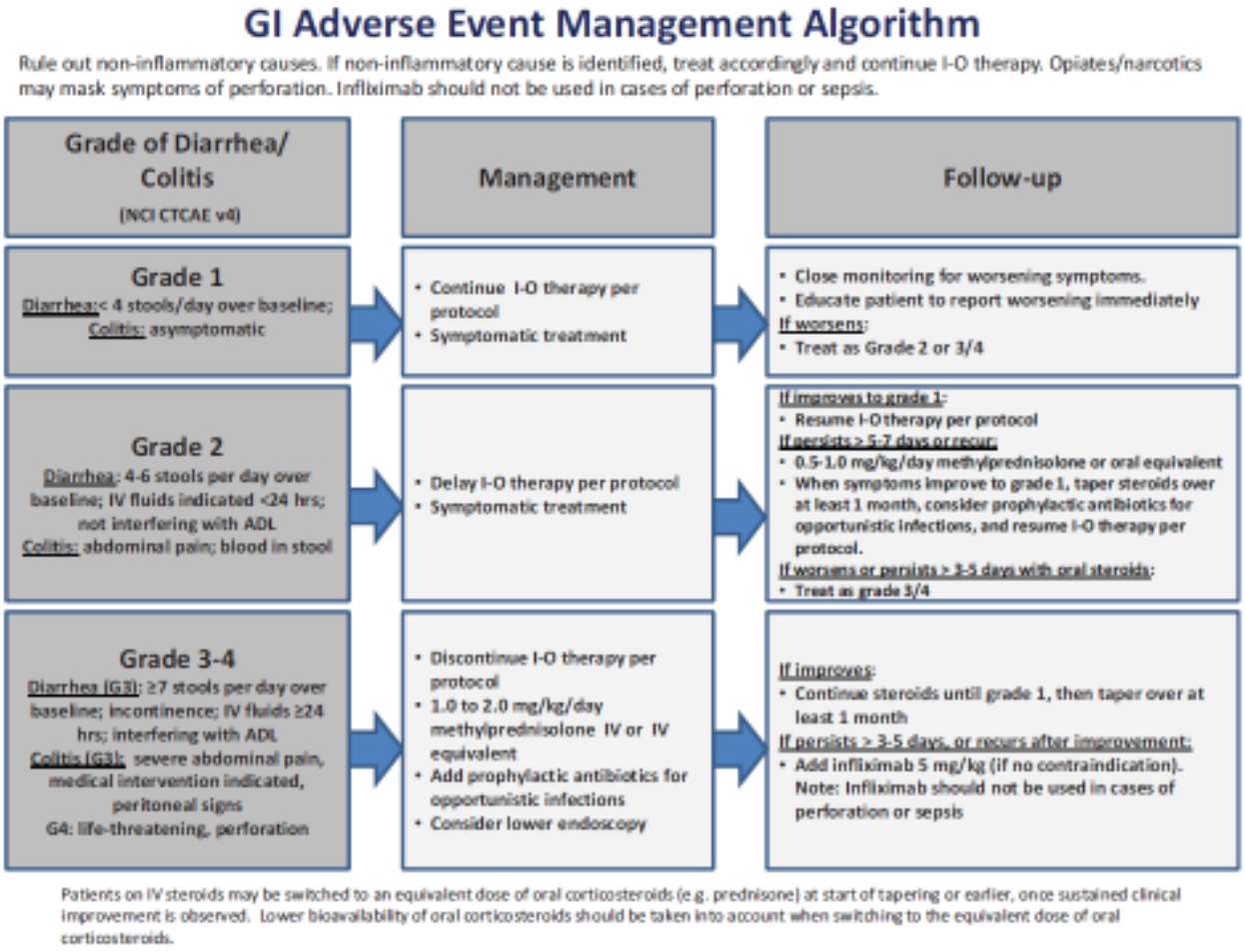
22. Pérez Gutthann, S.; García Rodríguez, L.A. The increased risk of hospitalizations for acute liver injury in a population with exposure to multiple drugs. Epidemiology 1993, 4, 496–501. [CrossRef] [PubMed]

23. García Rodríguez, L.A.; Ruigómez, A.; Jick, H. A review of epidemiologic research on drug-induced acute liver injury using the general practice research data base in the United Kingdom. Pharmacotherapy 1997, 17, 721–728. [PubMed]

24. De Abajo, F.J.; Montero, D.; Madurga, M.; García Rodríguez, L.A. Acute and clinically relevant drug-induced liver injury: A population based case-control study. Br. J. Clin. Pharmacol. 2004, 58, 71–80. [CrossRef]

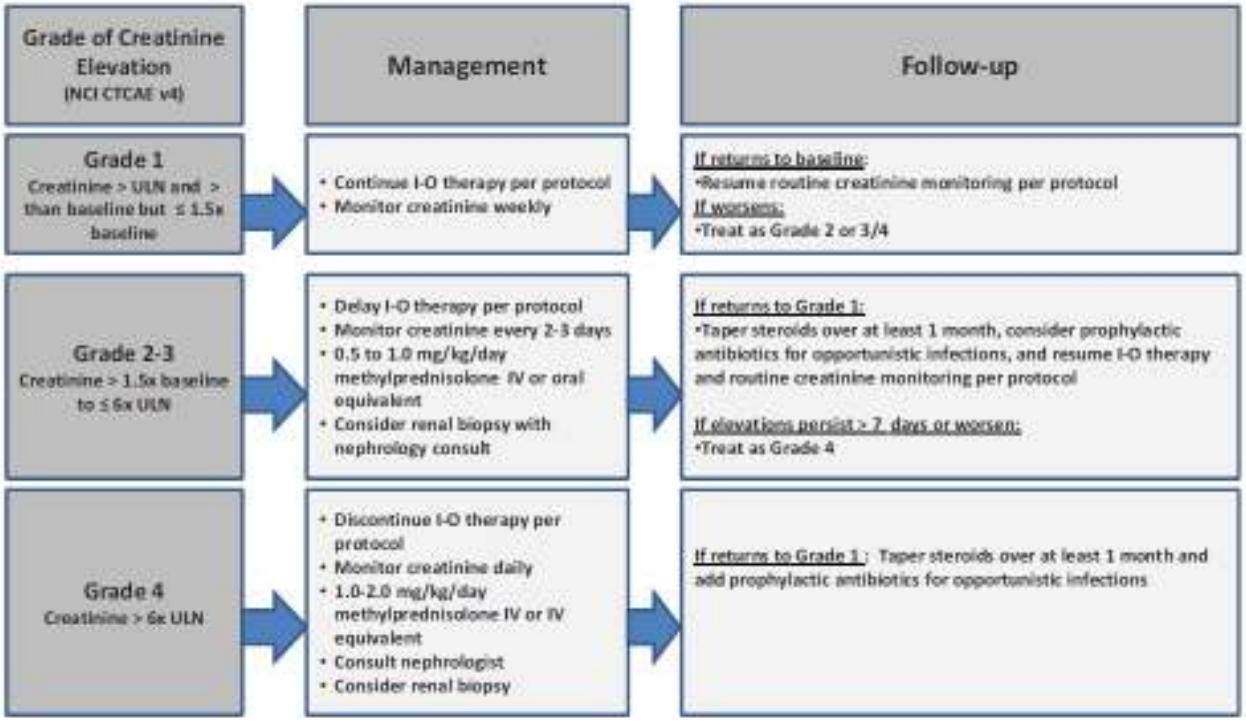
[PubMed]

Appendix 7.0 AE Management Algorithms



Renal Adverse Event Management Algorithm

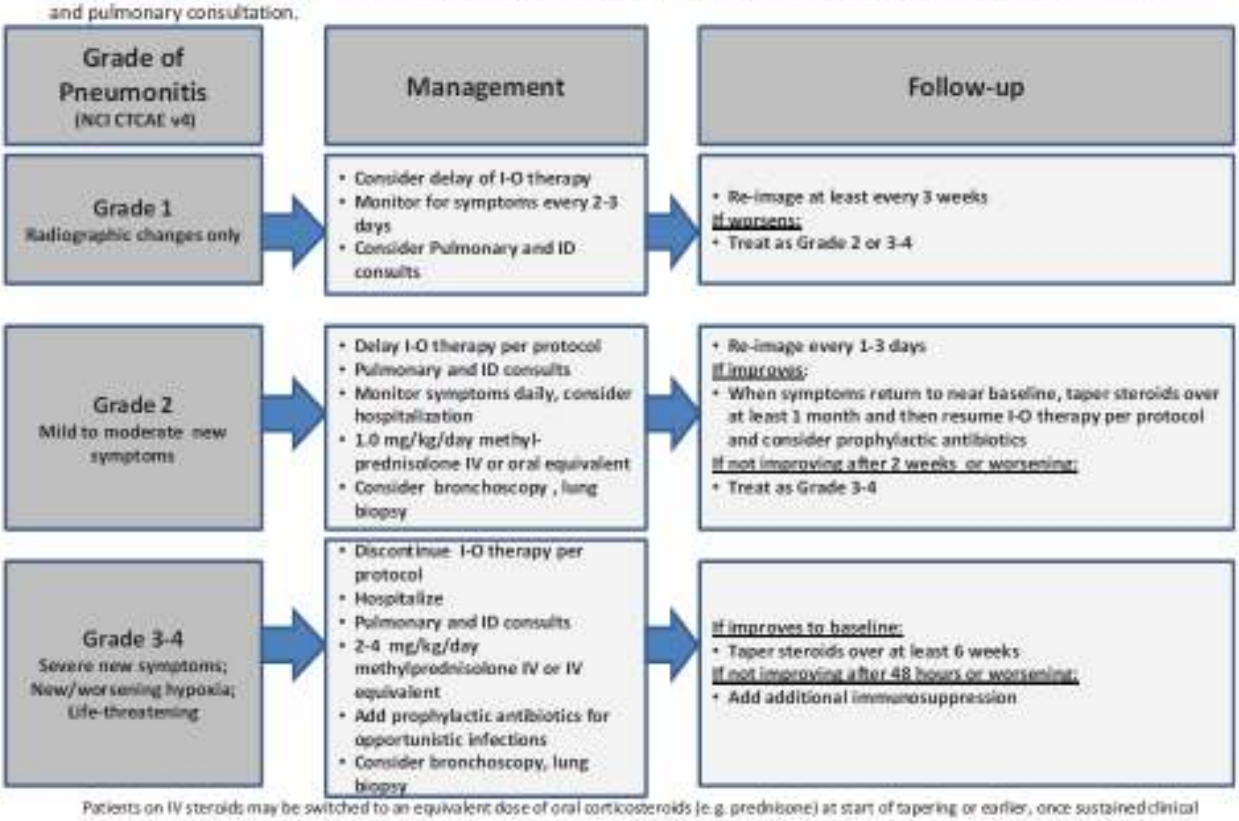
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



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

Pulmonary Adverse Event Management Algorithm

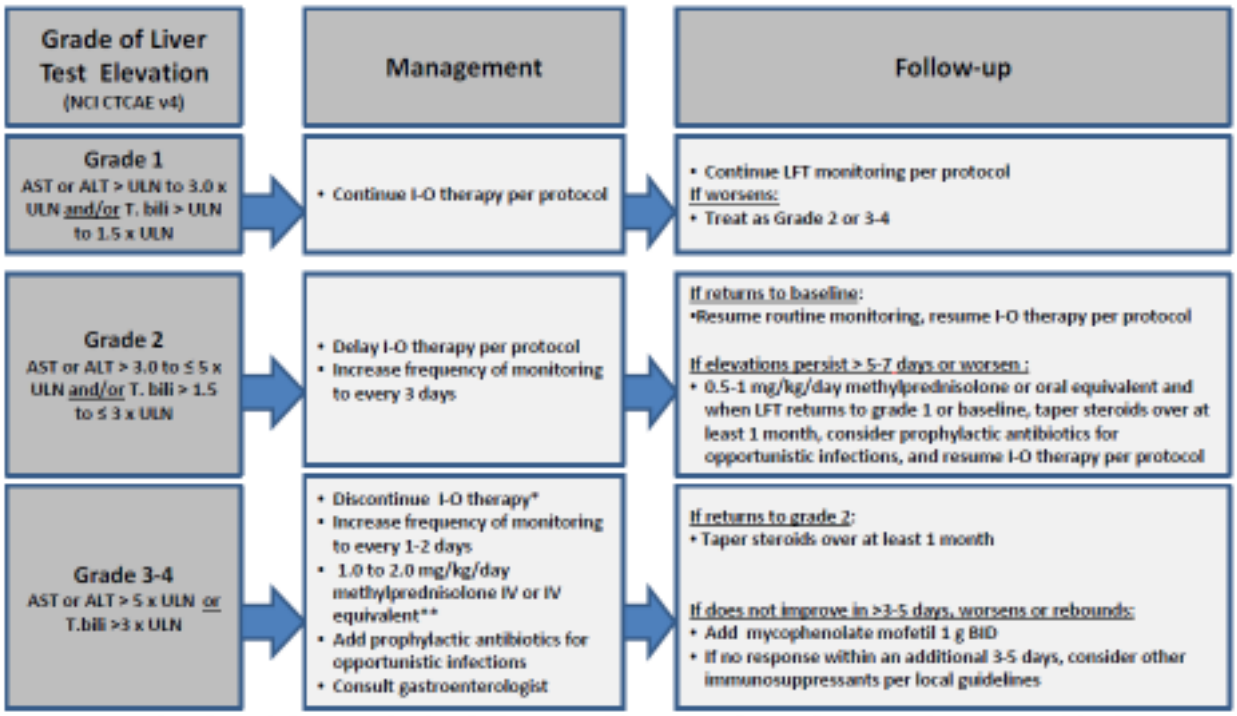
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



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

Hepatic Adverse Event Management Algorithm

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



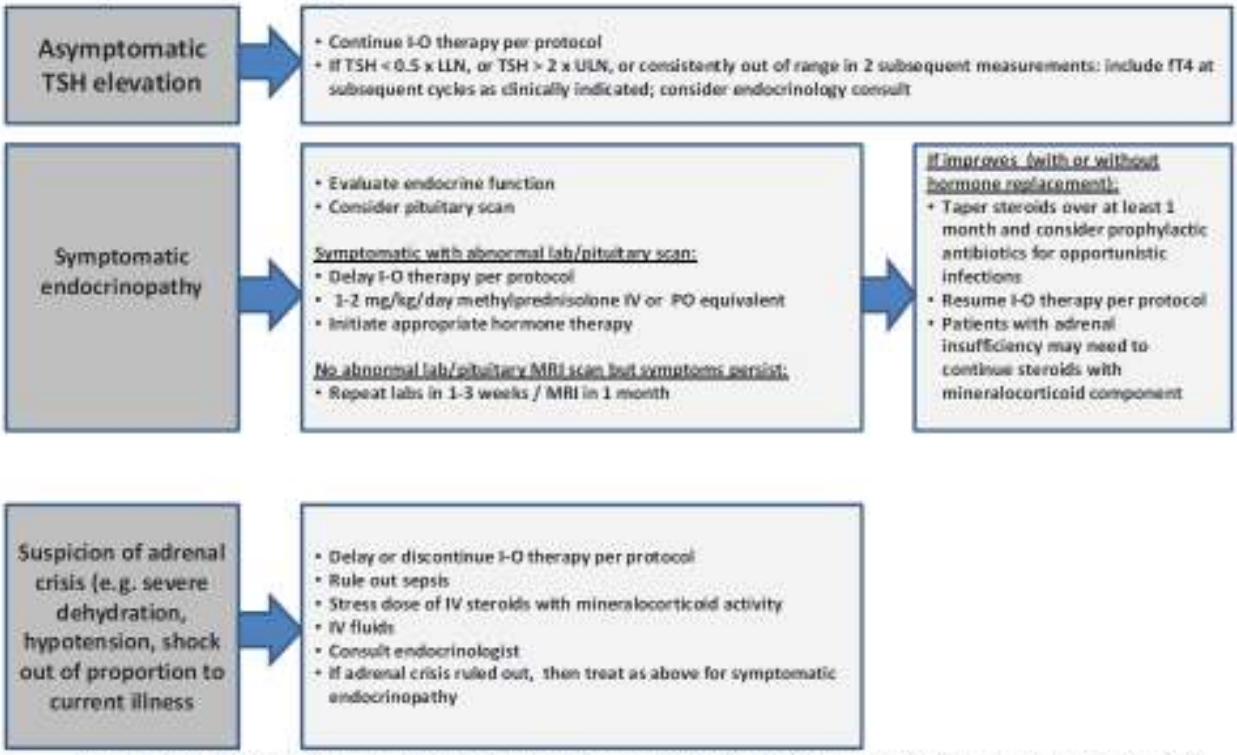
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of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

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

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

Endocrinopathy Management Algorithm

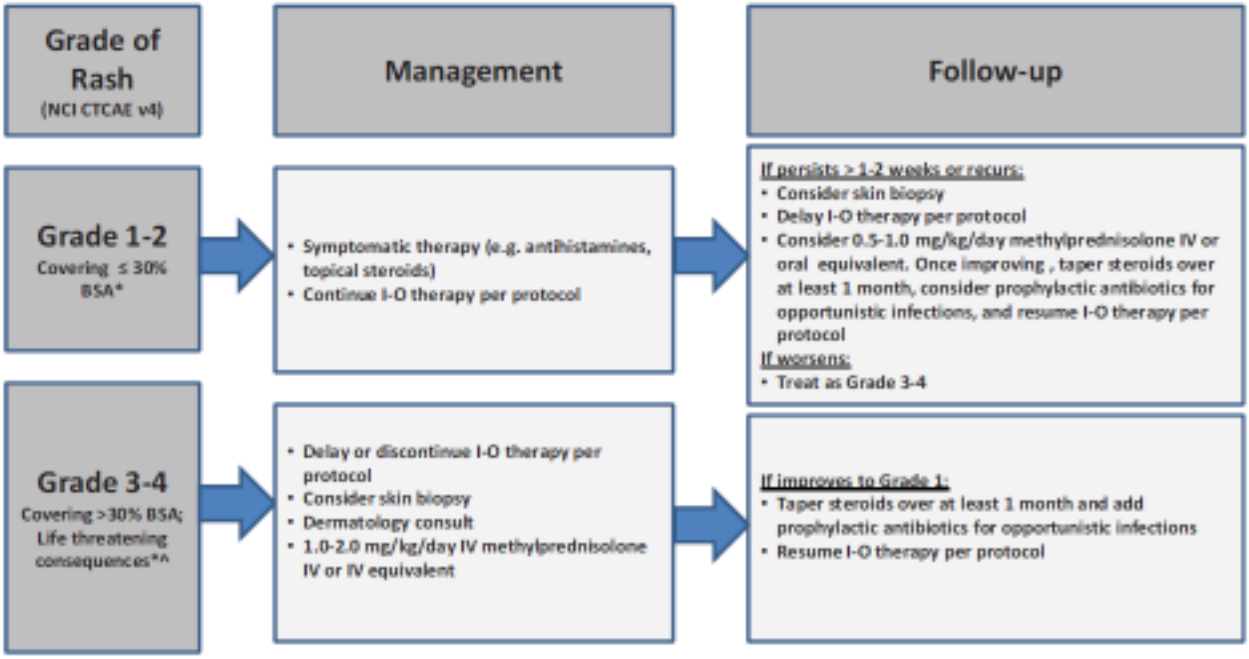
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



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

Skin Adverse Event Management Algorithm

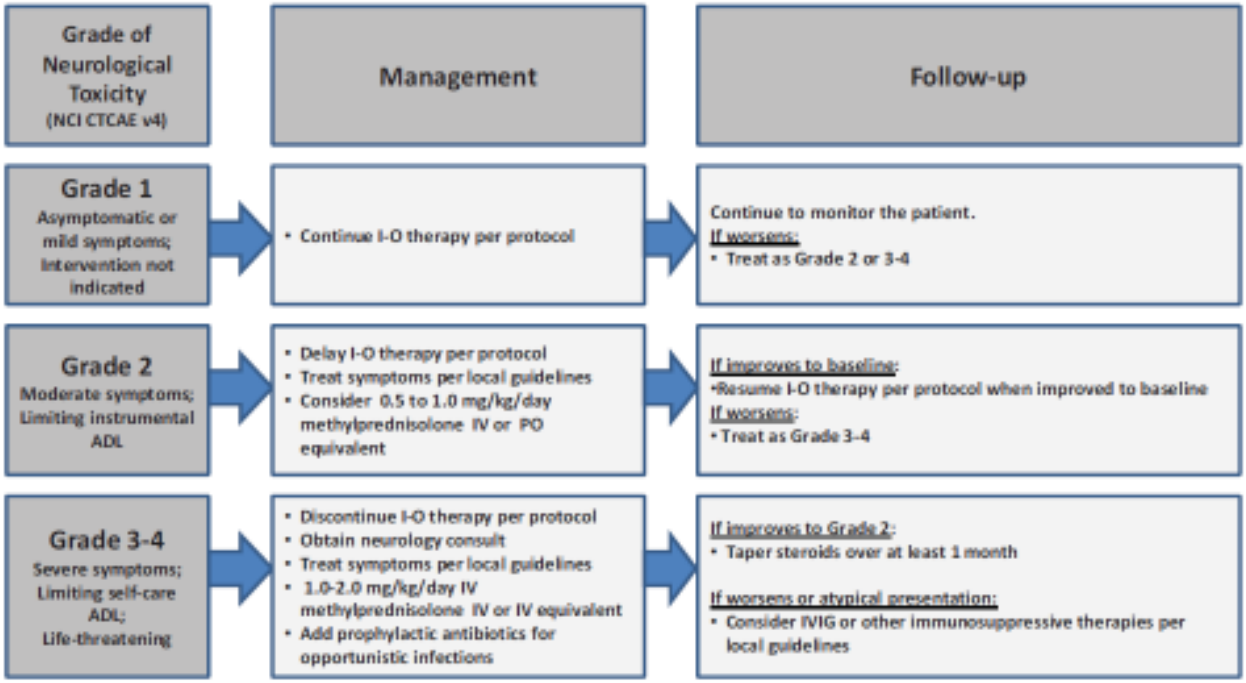
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



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

Neurological Adverse Event Management Algorithm

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



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

Appendix 8 Correlative Studies

Peripheral blood and tumor tissue-based assays: Blood and tumor specimens (when available) will be collected from each patient.

Tissue:
Up to ten unstained slides of 5 microns thickness or a block of tissue will be required to be sent if tissue is available. If the tissue is not available then Principal investigator permissions is required for enrollment. If the patient undergoes recent biopsy or resection then the more recent tissue is preferred. If the patient didn't undergo any recent surgery then the tissue from diagnosis can be used. Whole exome sequencing, transcriptome analysis, tumor mutational burden. Additional markers for immune function will be performed such as PDl- PD-1, PD-1L staining etc.

The tissue will be sent to
David Peereboom M.D.
Attn: Mary Mcgraw (Case 1317)
ND4-52 Lab,
Lerner Research Institute
9620 Carnegie Avenue, N Building, Cleveland, OH 44106

Collect 4-10ml green top tubes. Once the sample is collected it should be tubed to station 19, with a filled requisition (see Appendix 7).

Blood will be sent to the lab of Dr. C. Marcela Diaz-Montero for analysis.
Lerner Research 2111 E. 96th St. NE4-216 Cleveland, OH 44106 Attention: Pat Rayman
Provide advance notice by calling the lab at 216-444-5589 or emailing Pat Rayman at raymanp@ccf.org.

Methods:
Characterization of circulating immune cells: Frequencies of MDSCs, Tregs, $CD8^+$ T cells, $CD4^+$T and additional circulating immune cells will be determined by flow cytometry in both unfractionated blood and in purified PBMCs. PBMCs will be isolated from whole blood using the standard ficoll separation assay. Expression of immunomodulatory factors (PD-1, PD-1L, Lag3, Tim3, OX40, 41BB) on circulating immune cells will be also performed by flow cytometry.

Characterization of tumor immune infiltrate: Fresh tumor tissue will be digested to single cell suspension and analyzed by flow cytometry for frequencies of MDSCs, Tregs,

$CD8^+$ T cells, and $CD4^+$T cells. Expression of immunomodulatory factors (such as but not limited to PD-1, PD-1L, Lag3, Tim3, OX40, 41BB) on tumor infiltrating immune cells will also be assayed by flow cytometry.

Cytokine/Chemokine profile: Plasma will be isolated from whole blood and analyzed for levels of cytokines/chemokines involved in Th1 and Th2 responses. A multiplex system that measures 50+ analytes will be used.

Appendix 9 Laboratory Requisition

CASE 1317: A Randomized Phase 2 Open Label Study of Nivolumab plus Standard Dose Bevacizumab versus Nivolumab plus Low Dose Bevacizumab in Recurrent Glioblastoma (GBM)

Name_____
CCF#_____

Date_____ MD_____
Collected by_____ Time_____

Case IRB

Consented: Y N

PLEASE DRAW:

(4) 10 ml Green top (Sodium Heparin) tubes
Must fill tubes all the way
Mix/Invert 5-7 times after Draw
Send to Station 19
Attention Dr. Finke/Diaz Lab 444-5589 (20567)

(DO NOT REFRIGERATE)
Send this requisition with the sample

Appendix 10 NANO Scale

CA209-382 – A Randomized Phase 2 Open Label Study of Nivolumab plus standard dose Bevacizumab versus Nivolumab plus low dose Bevacizumab in Recurrent Glioblastoma (GBM)

Patient ID: _____ Date of Assessment: _____

Figure 1. Neurologic Assessment in Neuro-Oncology (NANO) Scale

Scoring assessment is based on direct observation and testing performed during clinical evaluation and is not based on historical information or reported symptoms. Please check 1 answer per domain. Please check "Not assessed" if testing for that domain is not done. Please check "Not evaluable" if a given domain cannot be scored accurately due to pre-existing conditions, co-morbid events and/or concurrent medications.

Date Assessment Performed (day/month/year):_____

Study time point (i.e. baseline, cycle 1, day 1, etc):_____

Assessment performed by (please print name): _____

Domains	Key Considerations
Gait 0 ☐ Normal 1 ☐ Abnormal but walks without assistance 2 ☐ Abnormal and requires assistance (companion, cane, walker, etc.) 3 ☐ Unable to walk ☐ Not assessed ☐ Not evaluable	• Walking is ideally assessed by at least 10 steps
Strength 0 ☐ Normal 1 ☐ Movement present but decreased against resistance 2 ☐ Movement present but none against resistance 3 ☐ No movement ☐ Not assessed ☐ Not evaluable	• Test each limb separately • Recommend assess proximal (above knee or elbow) and distal (below knee or elbow) major muscle groups • Score should reflect worst performing area • Patients with baseline level 3 function in one major muscle group/limb can be scored based on assessment of other major muscle groups/limb
Ataxia (upper extremity) 0 ☐ Able to finger to nose touch without difficulty 1 ☐ Able to finger to nose touch but difficult 2 ☐ Unable to finger to nose touch ☐ Not assessed ☐ Not evaluable	• Non-evaluable if strength is compromised • Trunk/lower extremities assessed by gait domain • Particularly important for patients with brainstem and cerebellar tumors • Score based on best response of at least 3 attempts
Sensation 0 ☐ Normal 1 ☐ Decreased but aware of sensory modality 2 ☐ Unaware of sensory modality ☐ Not assessed ☐ Not evaluable	• Recommend evaluating major body areas separately (face, limbs and trunk) • Score should reflect worst performing area • Sensory modality includes but not limited to light touch, pinprick, temperature and proprioception • Patients with baseline level 2 function in one major body area can be scored based on assessment of other major body areas

Visual Fields

- 0 ☐ Normal
- 1 ☐ Inconsistent or equivocal partial hemianopsia (≥quadrantopsia)
- 2 ☐ Consistent or unequivocal partial hemianopsia (≥quadrantopsia)
- 3 ☐ Complete hemianopsia
- ☐ Not assessed
- ☐ Not evaluable

- Patients who require corrective lenses should be evaluated while wearing corrective lenses
- Each eye should be evaluated and score should reflect the worst performing eye

Facial Strength

- 0 ☐ Normal
- 1 ☐ Mild/moderate weakness
- 2 ☐ Severe facial weakness
- ☐ Not assessed
- ☐ Not evaluable

- Particularly important for brainstem tumors
- Weakness includes nasolabial fold flattening, asymmetric smile and difficulty elevating eyebrows

Language

- 0 ☐ Normal
- 1 ☐ Abnormal but easily conveys meaning to examiner
- 2 ☐ Abnormal and difficulty conveying meaning to examiner
- 3 ☐ Abnormal. If verbal, unable to convey meaning to examiner. OR non-verbal (mute/global aphasia)
- ☐ Not assessed
- ☐ Not evaluable

- Assess based on spoken speech. Non-verbal cues or writing should not be included.
- **Level 1**: Includes word finding difficulty; few paraphasic errors/neologisms/word substitutions; but able to form sentences (full/broken)
- **Level 2**: Includes inability to form sentences (<4 words per phrase/sentence); limited word output; fluent but "empty" speech.

Level of Consciousness

- 0 ☐ Normal
- 1 ☐ Drowsy (easily arousable)
- 2 ☐ Somnolent (difficult to arouse)
- 3 ☐ Unarousable/coma
- ☐ Not assessed
- ☐ Not evaluable

- None

Behavior

- 0 ☐ Normal
- 1 ☐ Mild/moderate alteration
- 2 ☐ Severe alteration
- ☐ Not assessed
- ☐ Not evaluable

- Particularly important for frontal lobe tumors
- Alteration includes but is not limited to apathy, disinhibition and confusion
- Consider subclinical seizures for significant alteration