SUMMARY OF CHANGES

NCI Protocol #: Not applicable
DF/HCC Protocol #: 20-675

AM#	Section	Page(s)	Change
1.	N/A	N/A	N/A
2.	6.2 6.2 7.3 10.1 13.4	32 34 51 57-61 73-74	Added neurotoxicity specifics to DLT definitions Added dose modification for grade 2 neurotoxicity DLT Added neurotoxicity specifics to AE reporting Corrected Cycle Labels Added neurotoxicity Stopping Rule
3.	5 6 10 12	23-32 35 59 74-78	Clarified Safety Run-In Treatment Plan Clarified Drug Hold Information Clarified Safety Run-In Schedule Updated DSMC and Safety Run-In Plan
4.	1 1 3 4, 5 5 5, 6, 7 10 13	1 2 19, 21 23, 24, 33, 34 25-27 31-34, 55 63 75	Title revised Contact Information updated Allowing participants with no prior immunotherapy and revising radiotherapy washout Contact Information updated Updated infusion windows AE follow-up clarified Sampling Instructions Revised Sub-group analysis revised
5.			

NCI Protocol #: Not applicable

DF/HCC Protocol #: 20-675

TITLE: A Blinded, Randomized Phase 2 Study of Troriluzole in Combination with Ipilimumab and Nivolumab in Patients with Melanoma Brain Metastases

Sponsor-Investigator:
Ann W. Silk, MD, MS
Dana-Farber Cancer Institute
ann_silk@dfci.harvard.edu

Principal Investigator (PI):
Ann W. Silk, MD, MS
Dana-Farber Cancer Institute
ann_silk@dfci.harvard.edu

Other Investigators:
Ayal Aizer, MD
Elizabeth I. Buchbinder, MD
Daniel Cagney, MD
Rizwan Haq, MD, PhD
F. Stephen Hodi, MD
Megan Insco, MD, PhD
Eudocia Lee, MD
David Liu, MD, MPH, MS
Patrick Ott, MD, PhD

Statistician:
Anita Giobbie-Hurder, MS
Dana-Farber Cancer Institute
agiohur.ds@dfci.harvard.edu

Study Coordinator:
DFCI Melanoma
Group Inbox
Dana-Farber Cancer Institute
DFCIMelanomaCRCs@partnershealthcare.onmicrosoft.com
Fax: 617-582-8216

Responsible Research Nurses:
Amanda Livengood, RN
Dana-Farber Cancer Institute

Jill Gormley, RN
Dana-Farber Cancer Institute

Responsible Research Manager:
David Husselbee
Dana-Farber Cancer Institute
Telephone: 617-632-6704
Fax: 617-582-8216
David_husselbee@dfci.harvard.edu

NCI-Supplied Agents: None

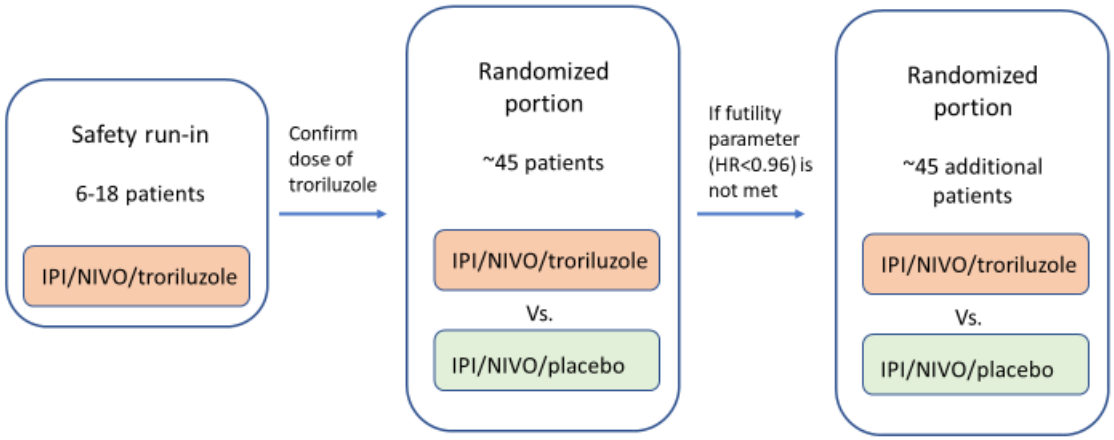
Other Agent(s):
Troriluzole, Biohaven Pharmaceuticals
Ipilimumab (NSC-732442), commercial supply
Nivolumab (NSC-748726), commercial supply

IND #: *153188*
IND Sponsor: Ann W. Silk, MD

Protocol Type / Version # / Version Date: Revised / Version #5 / Version date 11/15/2021

SCHEMA

Study Flow Diagram



Treatment Schema for Randomized Blinded Placebo-Controlled Trial

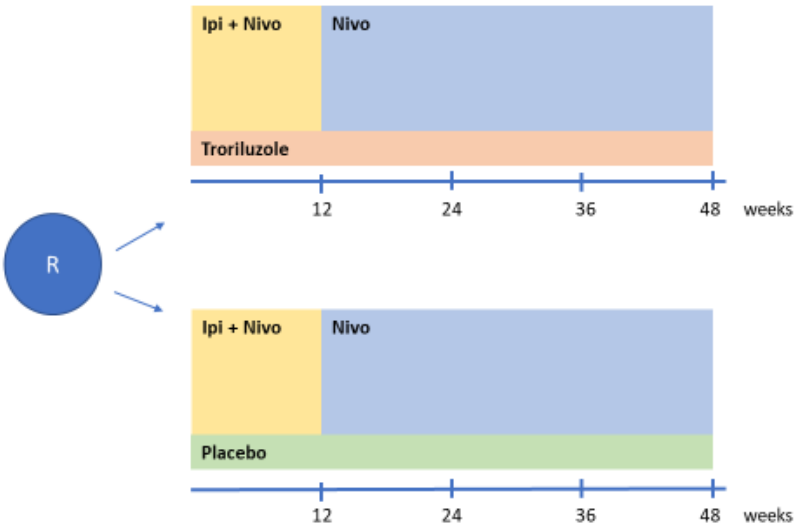


TABLE OF CONTENTS

SCHEMA 2

1. OBJECTIVES
   - 1.1 Study Design 6
   - 1.2 Primary Objectives 6
   - 1.3 Secondary Objectives 6
   - 1.4 Exploratory Objectives 7

2. BACKGROUND 7
   - 2.1 Melanoma brain metastases 7
   - 2.2 Glutamate Signaling in Melanoma 8
   - 2.3 Riluzole 8
   - 2.4 Troriluzole 11
   - 2.5 Ipilimumab and Nivolumab 14
   - 2.6 Radiation Therapy 15
   - 2.7 Rationale 16
   - 2.8 Correlative Studies Background 17

3. PARTICIPANT SELECTION 18
   - 3.1 Eligibility Criteria 18
   - 3.2 Exclusion Criteria 20
   - 3.3 Inclusion of Women and Minorities 21

4. REGISTRATION And Randomization PROCEDURES 21
   - 4.1 General Guidelines for DF/HCC Institutions 21
   - 4.2 Registration Process for DF/HCC Institutions 22
   - 4.3 General Guidelines for Other Investigative Sites 22
   - 4.4 Registration Process for Other Investigative Sites 22

5. TREATMENT PLAN 23
   - 5.1 Treatment Regimen 23
   - 5.2 Pre-Treatment Criteria 26
   - 5.3 Agent Administration 26
   - 5.4 End of Treatment 28
   - 5.5 Concomitant Medication and Supportive Care Guidelines 28
   - 5.6 Concomitant Brain-Directed Stereotactic Radiation therapy during the study 29
   - 5.7 Concomitant Surgery therapy during the study 30
   - 5.8 Criteria for Taking a Participant Off Protocol Therapy 30
   - 5.9 Duration of Follow Up 31
   - 5.10 Criteria for Taking a Participant Off Study 32
   - 5.11 Requests for Unblinding 32

6. DOSING DELAYS/DOSE MODIFICATIONS 34
   - 6.1 General considerations 34

6.2 Dose Limiting Toxicities 34
6.3 Troriluzole....35
6.4 Ipilimumab and Nivolumab 37
6.5 Treatment of Infusion Reactions Associated with Ipilimumab and/or Nivolumab....43
6.5 Treatment of Ipilimumab-Related Isolated Drug Fever....44
6.6 Monitoring and Management of Immune-mediated Adverse Reactions 45
6.7 Overlapping toxicities ("shared AEs") 48

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS 49
7.1 Expected Toxicities....49
7.2 Adverse Event Characteristics 53
7.3 Adverse Event Reporting....53
7.4 Reporting to the Food and Drug Administration (FDA) 55
7.5 Reporting to Hospital Risk Management....55
7.6 Routine Adverse Event Reporting 55

8. PHARMACEUTICAL INFORMATION....55
8.1 Troriluzole....55
8.2 Ipilimumab....58
8.3 Nivolumab....58

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES 58
9.1 Biomarker Studies....58
9.2 Laboratory Correlative Studies....59

10. STUDY CALENDAR 59
10.1 Scheduling windows 59

11. MEASUREMENT OF EFFECT....65
11.1 Antitumor Effect – Intracranial tumors and Extracranial tumors 65
11.2 Methods for Evaluation of Disease....67
11.3 Response Criteria: modified RECIST version 1.1 69
11.4 Duration of Response....71
11.5 Progression-Free Survival....72
11.6 Response Review 72

12. DATA REPORTING / REGULATORY REQUIREMENTS....72
12.1 Data Reporting 72
12.2 Data Safety Monitoring....73
12.3 Multi-Center Guidelines 73
12.4 Collaborative Agreements Language....73

13. STATISTICAL CONSIDERATIONS....73
13.1 Study Design/Endpoints....73
13.2 Sample Size, Accrual Rate and Study Duration 75
13.3 Stratification Factors....76

13.4 Interim Monitoring Plan 76
13.5 Analysis of Primary Endpoints 77
13.6 Analysis of Secondary Endpoints 77
13.7 Reporting and Exclusions 79

14. PUBLICATION PLAN 79

15. REFERENCES 80

APPENDIX A PERFORMANCE STATUS CRITERIA 83

APPENDIX B MULTI-CENTER GUIDELINES 84

1. Introduction 84
1.1 Purpose....84

2. GENERAL ROLES AND RESPONSIBILITIES 84
2.1 Coordinating Center....84
2.1 External Site....85

3. DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS....86
3.1 Protocol Revisions and Closures 86
3.2 Informed Consent Requirements 86
3.3 IRB Re-Approval....87
3.4 DF/HCC Multi-Center Protocol Confidentiality 87
3.5 Participant Registration and Randomization 87
3.6 Initiation of Therapy 88
3.7 Eligibility Exceptions....88
3.8 Data Management 88
3.9 Protocol Reporting Requirements....88
3.10 Reporting Procedures....89
3.11 Guidelines for Processing IND Safety Reports 89

4. MONITORING: QUALITY CONTROL 89
4.1 Ongoing Monitoring of Protocol Compliance 89
4.2 Monitoring Reports....89
4.3 Accrual Monitoring....90

5. AUDITING: QUALITY ASSURANCE 90
5.1 DF/HCC Internal Audits....90
5.2 Audit Notifications....90
5.3 Audit Reports....90
5.4 External Site Performance 90

APPENDIX E External Site Subject Registration Form 91

APPENDIX F. PILL DIARY 93

1. OBJECTIVES

1.1 Study Design

We propose a multi-center, blinded, randomized, phase II signal-detection trial with safety run-in to assess the efficacy and safety of adding troriluzole to ipilimumab + nivolumab in patients with melanoma that has metastasized to the brain.

There will be a Safety Run-in, which will be a single-arm, open-label 3+3 design in which all subjects will be treated with ipilimumab + nivolumab + troriluzole (INT). Following the Safety Run-in (Part I), all participants will be enrolled on the double-blind randomized portion of the study (Part II). Subjects will be randomly assigned in equal proportions to induction therapy with ipilimumab + nivolumab + placebo (INP) or ipilimumab + nivolumab + troriluzole (INT). Subjects assigned to the troriluzole vs. placebo arm will continue troriluzole or placebo in the maintenance period with nivolumab. The randomization will be stratified by prior therapy with BRAF-directed therapy (yes/no) and prior therapy with anti-PD-1 treatment (yes/no).

1.2 Primary Objectives

The primary objective is to assess the efficacy and safety of adding troriluzole to ipilimumab + nivolumab in patients with melanoma that has metastasized to the brain.

The primary endpoint of the study is global progression-free survival (PFS) in patients with melanoma brain metastases who are treated with troriluzole + ipilimumab + nivolumab + (INT) vs. ipilimumab + nivolumab (INP). The proportions at 6 months will be of particular interest.

Herein, the term "PFS" refers to PFS in both the intracranial and extracranial compartments (global).

1.3 Secondary Objectives

The secondary objectives are to evaluate other important clinical outcomes, including:

- overall survival
- intracranial response rate (RR) and PFS
- extracranial RR and PFS
- safety (number of participants experiencing adverse events, particularly serious neurologic events such as seizure, MRI-defined intracranial hemorrhage, radiation necrosis)
- tolerability (number of induction cycles administered, number of maintenance cycles administered)
- use of corticosteroids for management of symptomatic cerebral edema (number of participants)
- use of corticosteroids for management of immune related adverse events (number of participants who require prednisone ≥1 mg/kg or equivalent)

- frequency of clinically-indicated stereotactic radiation therapy to the brain on study (number of patients, number of instances and number of lesions irradiated)
- frequency of clinically-indicated surgical intervention to the brain on study (number of participants)

1.4 Exploratory Objectives

- Determine associations between BRAF/NRAS mutation status and response endpoints, as well as tissue biomarker profiles between paired tissues from extracranial and intracranial metastases from individual patients, where available
- Determine associations between peripheral blood immune cell subpopulations (which may include but is not limited to T-cell, NK, B-cell, MDSC, and serum soluble factors) with clinical endpoints and/or the occurrence of adverse events

2. BACKGROUND

2.1 Melanoma brain metastases

Brain metastases are a key complication of melanoma because 44% of patients with metastatic melanoma will develop symptomatic brain metastases, and intracranial disease accounts for 20% to 54% of deaths in patients with melanoma.[1] Prognosis is poor, and historical estimates of survival range from 3 to 13 months despite radiation therapy (RT), which includes stereotactic radiotherapy (SRT; also known as stereotactic radiosurgery or SRS) and whole brain radiation therapy (WBRT). The most common solid tumors to form brain metastases are lung cancer, breast cancer, and melanoma. Melanoma is unique in that it is common for there to be multiple synchronous brain metastases rather than a single brain metastasis.

Brain metastases have distinct metabolic and immunologic features as compared to extracranial metastases. There is increased dependence on the PI3K pathway and increased dependence on glutamine and oxidative phosphorylation. Recently, Davies and colleagues from M.D. Anderson performed RNA sequencing on patient-matched resected melanoma brain metastases and extracranial metastases. Compared with patient-matched extracranial metastases, brain metastases exhibited substantial immunosuppression and elevated levels of oxidative phosphorylation gene expression and metabolites.[2]

Systemic therapy for melanoma has improved dramatically since 2011 with the availability of immunotherapy agents, including the immune checkpoint inhibitor antibodies ipilimumab, pembrolizumab and nivolumab. The PD-1 inhibitors pembrolizumab and nivolumab are more effective and less toxic than the CTLA-4 inhibitor ipilimumab, and they have a response rate of 30-40% when given as single agents. Dual CTLA-4 and PD-1 checkpoint inhibition (ipilimumab and nivolumab) is approved in combination for melanoma with a response rate of 56%. Patients with untreated, uncontrolled brain metastases were excluded from these early studies.

The importance of systemic therapy for the treatment of melanoma brain metastases has recently been demonstrated in a study using dual checkpoint blockade with IPI/NIVO.[3] In neurologically

asymptomatic patients with small volume brain metastases and no previous systemic therapy, the combination was shown to be an active regimen with a response rate of 48%.[3] The response rate in symptomatic patients was far lower. The vast majority of patients have concordant responses in their intracranial and extracranial disease. Brain-directed stereotactic radiation therapy of one lesion was allowed on study. However, as the PFS at 12 months was only 57%, there is still an unmet clinical need in this population.

The clinical management of a single metastasis with craniotomy and/or brain-directed stereotactic radiation is supported by the literature and has been the standard of care for nearly twenty years. Prior to the approval of immune checkpoint inhibitors, radiation therapy, in the form of WBRT or SRT, was the considered the mainstay of treatment. In randomized controlled trials, WBRT failed to demonstrate benefits in disease control or survival,[4,5] and the field has shifted away from using WBRT by itself or following SRT.[6] Since 2012, a large amount of retrospective data and some prospective data has emerged combining immune checkpoint inhibitors with SRT,[7-14] and concurrent immune checkpoint inhibitors + SRT is now considered standard of care. Complications of brain metastases including perilesional edema, intralesional hemorrhage, and radiation necrosis have been reported;[15] however, there is no clear evidence that the frequency of these complications is higher than expected, and the impact of these events must be weighed against the complications arising from untreated, progressive brain metastases.

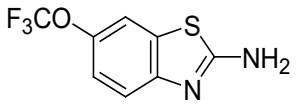
2.2 Glutamate Signaling in Melanoma

Glutamate (the anion of the amino acid called glutamic acid) is the most abundant excitatory neurotransmitter in the brain. The glutamatergic system also plays a key role in tumor biology. The Metabotropic Glutamate Receptor 1 (GRM1) is expressed in 60-100% of human melanomas. GRM1-expressing melanoma cell lines release elevated levels of glutamate, which is the natural ligand for GRM1, thus establishing autocrine loops and constitutive activated GRM1. Activation of GRM1 leads to a dual signaling cascade that stimulates both MAPK and PI3K pathways. Antagonizing glutamate receptors in cell lines from various human tumors including melanoma, colon adenocarcinoma, breast, glioma, and lung carcinoma has been reported to inhibit their growth. Glutamate signaling may be important in carcinomagenesis, as ectopic expression of GRM1 is sufficient for the spontaneous development of a metastatic murine-form of melanoma in a transgenic mouse model.[16,17]

GRM1 signal transduction appears to induce a suppressive tumor microenvironment. GRM1 signal transduction in melanoma results in increased tumor-derived exosome production,[18] and increased expression of the immune suppressing factors M-CSF and CCL2, which promoted suppressive tumor-associated M2 macrophages. Over-expression of M-CSF and its receptor, CSF-1R, in tumors has been associated with a poor prognosis and M-CSF and CCL4 have also been implicated in mobilization of tumor-associated macrophages (TAMs) into the tumor microenvironment. In a laboratory model, GRM1 activation leads to inhibition of release of exosomes containing MCSF and CCL-2, which create an immunosuppressive tumor microenvironment by promoting TAMS.[19,20]

2.3 Riluzole

Riluzole is an FDA-approved medication for the debilitating neurologic disease amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease. The standard dose used clinically for ALS is 50 mg PO bid. The chemical structures of riluzole is shown below.



Riluzole, **1**

Figure 2.1. Chemical structure of riluzole.

Preclinical data. Following treatment with riluzole, the Chen lab observed a reduction in the number of viable cells in two different GRM1-positive cell lines (C8161 and SKMEL187) while two different GRM1-negative human melanoma cell lines (UACC930 and C81-61) or normal human melanocytes (HEM) were less sensitive under similar conditions. They demonstrated a correlation between a decrease in released glutamate and the number of viable cells in riluzole-treated C8161 cells, showing that high glutamate release may promote tumor cell growth.[17,21]

The cystine-glutamate amino acid antiporter (xCT) is an obligate transporter that exchanges glutamate for cystine at 1:1 ratio, meaning for every one molecule of cystine that is pumped in, one molecule of glutamate is pumped out into the extracellular space. Once transported intracellularly, cystine is reduced into 2 molecules of cysteine, the rate-limiting precursor of glutathione (GSH) synthesis. In normal melanocytes, transport of cystine by xCT is utilized for cell proliferation, glutathione production, and protection of cells from oxidative stress. In melanoma cell lines, riluzole stimulates the expression of xCT, inhibiting the release of glutamate into the extracellular space.[22]

In melanoma cell lines, inhibition of GRM1 blocks exosomal release of CCL4 and CSF1 (M-CSF), which normally recruit suppressive macrophages and exclude lymphocytes from infiltrating the tumor. This results in increased apoptotic death and pleotropic effects on the tumor microenvironment, including a decrease in the numbers of M2-type (suppressive) TAMs, myeloid-derived suppressor cells (MDSCs) and regulatory T cells (T regs), and an increase in the numbers of tumor-infiltrating lymphocytes (TILs).

Clinical trials. The group at Rutgers Cancer Institute of New Jersey evaluated riluzole for potential anti-cancer activity in Phase 0 and 2 clinical trials for advanced Stage III and IV melanoma, treating 25 patients in total. All of the patients (100%) had GRM1-positive tumors (100%).

In the Phase 0 trial, riluzole (100 mg PO BID) was given to patients with advanced but resectable melanoma for two weeks, followed by surgical resection and tissue collection for pharmacodynamic analysis.[23] Four out of 12 patients (34%) had a significant decrease in pAKT and/or pERK in post-treatment tumor samples as compared with their paired pretreatment sample. The same 4 patients had metabolic responses seen on FDG–PET scans despite a short duration of treatment (14 days) as seen in Figure 2.2.

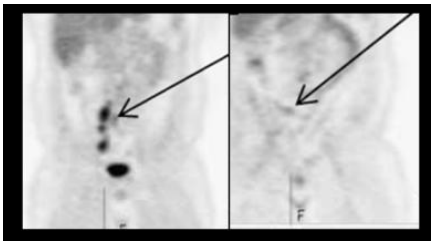


Figure 2.2. A representative FDG-PET scan of one of the four patients that had a radiologic and metabolic response to 2 wk of riluzole administration in the Phase O trial. Pretreatment FDG-PET scan (left) and post-treatment scan (right) done 2 wk after the start of riluzole. There is a large decrease in the number of FDG-avid lymph nodes seen in the post-treatment scan, and the remaining node seen in the right panel has decreased in intensity by >40%. The three other patients who had metabolic responses had similar FDG-PET scan results.

In the Phase 2 trial, there were no objective responses observed, but 6/13 patients who initially had rapidly progressive disease achieved stable disease and 4 of those patients maintained stable disease for 23-56 weeks. Riluzole was well-tolerated. There were no grade 4 or 5 adverse events, and grade 3 dizziness was seen in 5 patients (38%) but only one subject had to discontinue therapy due to dizziness. Pharmacokinetic analysis showed high inter-patient variability, consistent with prior riluzole studies. Markers of angiogenesis (VEGF, IL-8) were suppressed in the post-treatment samples in 4/8 (50%) and 3 of them had clinical benefit (stable disease).[24] Patients who had BRAF-mutated and B-RAF wild type tumors benefitted.

Immune effects. Exploratory correlative studies demonstrated a pattern suggesting that the patients who had clinical benefit from riluzole treatment displayed multiple downstream markers of GRM1 signaling inhibition, including suppression of MAPK, PI3K/AKT, and markers of angiogenesis. Additionally, two patients (#1 and #11) who derived clinical benefit from riluzole treatment displayed an increase in CD45+ leukocytes at the tumor-stromal interface (Figure 2.3a). Two patients who did not benefit from treatment did not show suppression of PI3K/AKT, angiogenesis, nor an increase in CD45+ cells at the interface (Figure 2.3b). The increase in leukocytes at the interface, or "active edge," of the tumor may be mediated by inhibition of exosomes containing MCSF and CCL-2, which normally recruit tumor-associated macrophages (TAMs) and exclude tumor infiltrating lymphocytes (TILs).

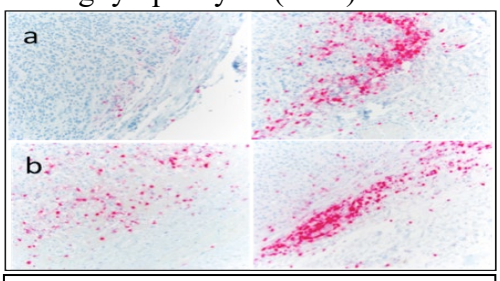


Figure 2.3a. CD45 staining on fresh paired tumor biopsies from 2 patients with stable disease (SD). a) CD45 from patients 1 (a) and 11 (b), before (left) and after (right) riluzole treatment, showing an increase in CD45+ cells in the tumor/stroma interface.

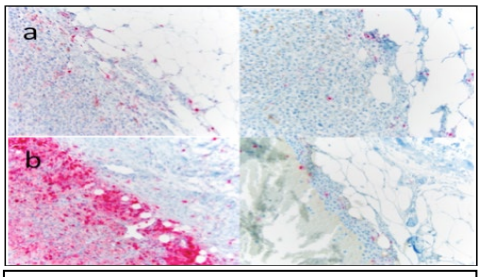


Figure 2.3b. CD45 staining on fresh paired tumor biopsies from 2 patients with progressive disease (PD). a) CD45 from patients 6 (a) and 8 (b), before (left) and after (right) riluzole, showing a decrease in intratumoral CD45+ cells after treatment.

Therefore, the pattern of the correlate studies outcomes suggests that the patients who had clinical benefit from riluzole treatment displayed multiple downstream markers of GRM1 signaling inhibition, including suppression of MAPK, PI3K/AKT, and markers of angiogenesis,

as well as an increase in leukocytes at the tumor-stroma interface. Put together, these factors suppress the growth of tumor cells and blood vessels and allow immune infiltrate into the edge of the tumor.

These correlative data are consistent with the mounting evidence demonstrating that anti-angiogenic agents can reprogram the immune milieu of the tumor microenviroment to improve the effectiveness of checkpoint blockade.[25] Bevacizumab treatment was associated with a post-treatment increase in T-cell infiltration in the tumor and increased MHC-Class I expression in patients with renal cell carcinoma and it improved the efficacy of the immune checkpoint inhibitor atezolizumab.[26] The strategy of combining an angiogenesis inhibitor with an immune checkpoint inhibitor has now been validated in several clinical trials and has led to two combinations approvals, pembrolizumab plus lenvatinib for endometrial cancer[27] and axitinib plus pembrolizumab or avelumab for first-line treatment of renal cell carcinoma.[28,29]

2.4 Troriluzole

i. Overview

Mechanism of action. Troriluzole (formerly known as FC-4157, BHV-4157, and trigriluzole) is an investigational tri-peptide prodrug of riluzole with improved PK/PD properties. Riluzole was FDA-approved for ALS in 1995. The mechanism of action of troriluzole, which is rapidly cleaved into riluzole, is not fully understood, is thought to be entirely due to the action of riluzole which blocks the release of glutamic acid and up-regulates the excitatory amino acid transporter EAAT2. Interruption of GRM1-mediated signaling by limiting the available ligand, glutamate, leads to intracellular build-up of glutamate. The build-up decreases the exchange of glutamate and cystine, which limits the availability of cysteine, a key component of L-Glutathione (GSH) synthesis. This process results in reduced intracellular GSH and enhanced cellular stress, genome instability and DNA damage.

Pharmacologic advantages. Blood levels of riluzole are highly variable upon oral administration of riluzole due to its metabolism by a particular cytochrome P450 enzyme, Cyp1A2, which is variably expressed. We have discovered a novel Type IIb prodrug of riluzole, troriluzole, that minimizes this variable metabolism, readily releases the drug riluzole into the blood stream when given orally to mice, rats, cynomolgus monkeys and humans and is >20X more potent than riluzole itself in a mouse melanoma xenograft model.

Highlights of the prodrug troriluzole include:

- Troriluzole is stable in simulated gastric fluid (SGF) and simulated intestinal fluid with stability half-lives of >60 and 98 min respectively.
- Troriluzole has adequate solubility (247 ug/mL) in a standard aqueous oral delivery vehicle (aqueous 2% Tween 80/0.5% methylcellulose) and high cellular permeability with no efflux potential.
- Troriluzole is highly stable in mouse (MLM) and human (HLM) liver microsomes with half-lives greater than 1 h in both species. Riluzole is metabolized to N-hydroxyriluzole in HLM containing high activity Cyp1A2 with a half-life of <30 min, but is stable to HLM with low activity of Cyp1A2.

- Troriluzole is actively transported by the PepT1 transporter.

Non-oncologic indications under investigation. Troriluzole is currently under investigation in phase 3 trials for spinocerebellar ataxia and generalized anxiety disorder. It is also in Phase 2 studies for Alzheimer's dementia and obsessive-compulsive disorder. In December 2019, Biohaven Pharmaceuticals announced that troriluzole successfully advanced past the interim futility analysis in the pivotal phase 2/3 Alzheimer's disease study, "T2 PROTECT AD."

ii. Supporting Preclinical Data for Troriluzole

Troriluzole is highly effective in reducing melanoma tumor growth in a mouse xenograft experiment carried out for 14 days. In this experiment, nude mice were injected with melanoma cells (C8161 line) and developed tumors that were visible after one week. A dose of troriluzole corresponding to 20X less riluzole being formed, was as active as riluzole itself, showing a >20X increase in potency in this in vivo model of melanoma (Figure 2.4).

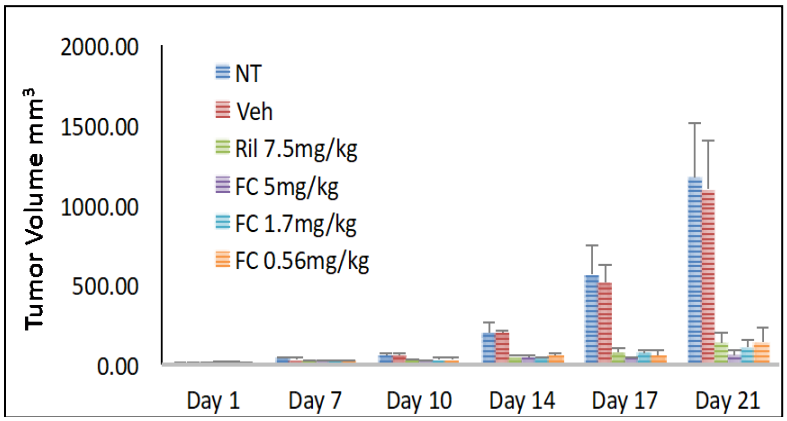


Figure 2.4. Trogriluzole ("FC", 1.7 mg/kg) exhibits an increase in potency when compared to riluzole (7.5 mg/kg) in a mouse xenograft model of melanoma. C8161 human melanoma cells (10^6) were inoculated in the flanks of nude mice and when the tumor volume reached 40 mm^3 the mice were divided randomly into groups with similar tumor volumes: No treatment (NT), vehicle (Veh, DMSO), riluzole (Ril, 7.5 mg/kg), Troriluzole (FC) at 0.56 mg/kg, 1.7 mg/kg and 5 mg/kg. Mice were treated daily by oral gavage. Tumor volume (mm^3) mean ±SD of 12 mice/group, P <0.001, for riluzole or troriluzole groups (regardless of dosage) compared to NT/Veh.

iii. Supporting Preclinical Data for Troriluzole + PD-1 Inhibition

Combination study in mouse model of melanoma. Very few preclinical animal studies have been carried out in immunotherapy due to lack of tumor growth in immunocompetent animal models. Many investigators have used the syngeneic mouse B16 melanoma model in C57BL/6 mice widely over the years. In B16 preclinical studies, combining CTLA-4 and PD1 blockade has been shown to increase CD8+cytotoxic and CD4+ helper T cell populations and to promote rejection of B16 tumor allografts (compared to either one alone). In our studies, we isolated several stable clones (MASS) derived from immortalized C57BL/6 murine melanocytes (Melan-A) transfected with exogenous murine GRM1 cDNA. MASS clones were very tumorigenic in both immune-deficient nude mice and immune-competent syngeneic C57BL/6 mice, while the parental Melan-A cells and control clones transfected with empty vector, MelanA-Vec, were not tumorigenic even after several months. GRM1 receptors in MASS clones are responsive to agonists/antagonists of GRM1,

suggesting a functional receptor.

We found that administering riluzole, troriluzole (FC-4157) or anti-PD-1 alone initially had no impact on inhibition of tumor outgrowth in this immunocompetent model. However, by Day 28, the combinations with anti-PD-1 demonstrated good tumor control (Figure 2.5).

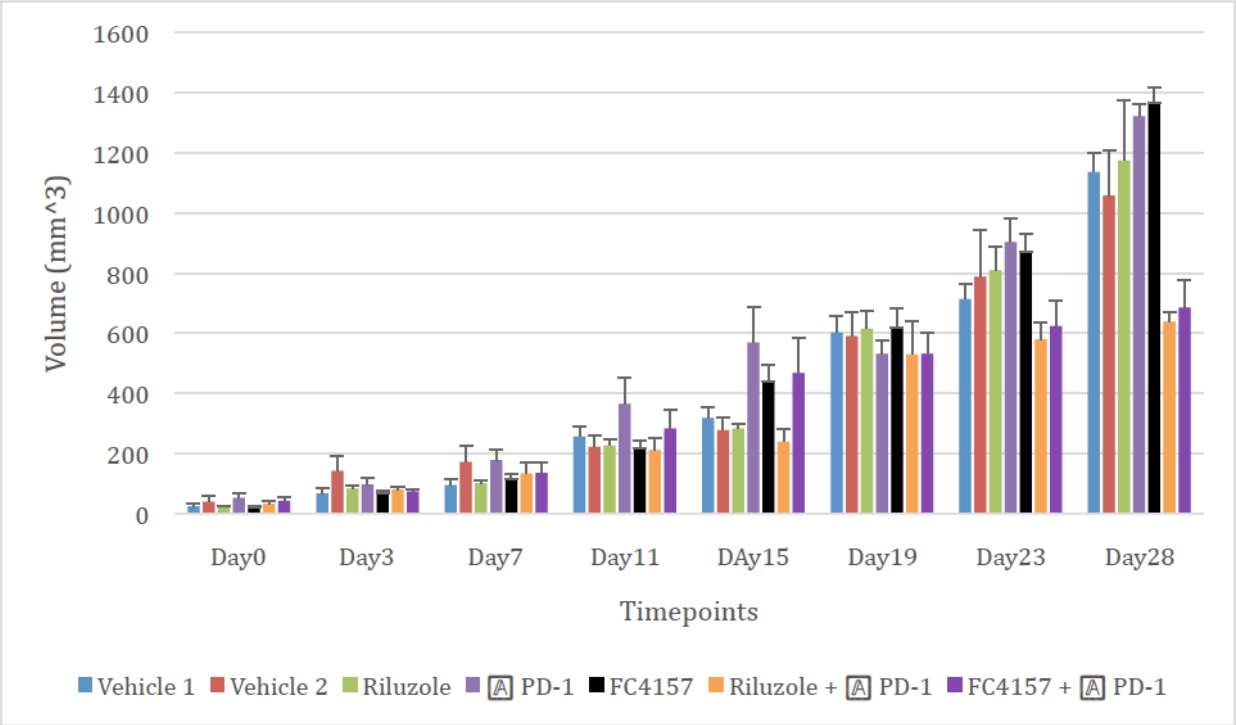


Figure 2.5. Troriluzole is active at Day 28 in combination with anti-PD-1 antibody in a MASS20 GRM-1 allograft mouse model, giving a similar response at ~10X less molar concentrations compared to riluzole itself. 10^5 MASS20 cells were inoculated into flanks of C57BL/6 mice. When the tumor volumes reached ≈40 mm^3, we randomly divided the tumor-bearing mice into vehicle 1 (DMSO), vehicle 2 (control rat IgG), riluzole (10 mg/kg) alone, anti-PD-1 (100 μg/mouse/injection) alone, troriluzole (1.7 mg/kg) alone, combination of riluzole and anti-PD-1, and combination of troriluzole and anti-PD-1. The treatment was terminated at 25 days after start of the treatment as day 1.

<u>The role of exosome inhibition in the immunologic mechanism of action of troriluzole</u>. Exosomes are naturally occurring small membrane enclosed microvesicles generated constitutively and released by fusion with the cell membrane by various cell types and more frequently by tumor cells. Hood et al. suggested an "exosomal messenger system" that facilitate communication within the local tumor microenvironment and is instrumental in melanoma cell dissemination and early events in metastasis. Studies show that melanoma exosomes "educate" bone progenitor cells to support tumor cell growth and metastasis, alter macrophage and dendritic cell functions, sometimes call "preparing the metastatic niche." In a laboratory model, GRM1 activation leads to inhibition of release of exosomes containing MCSF and CCL-2,[20] which create an immunosuppressive tumor microenvironment by promoting tumor-associated macrophages. Our preliminary results suggest that treatment with riluzole reduces exosome levels in vivo (Chen lab, unpublished data).

iv. Supporting Clinical Data for Troriluzole + PD-1 Inhibition

Given the hypothesis that troriluzole treatment inhibits GRM1 signaling, thereby inhibiting release of exosomes, decreasing TAMs, and allowing lymphocytes into the tumor-stromal interface, and the compelling preclinical data the troriluzole enhances the antitumor activity of PD-1 immune checkpoint inhibitor, a phase Ib trial was conducted at a single site. The combination of troriluzole and nivolumab was found to be well tolerated. We enrolled 14 patients with advanced solid tumors (melanoma = 3, NSCLC = 3, renal cell cancer = 2, NSCLC = 2, head and neck cancer = 2). Eleven patients had prior therapy with anti-PD-1 or anti-PD-L1. Patients were exposed to troriluzole doses from 140mg to 560mg total daily dose. Dose limiting toxicities (DLT) occurred in 3 patients which were characterized as 1) intolerable nausea in a patient who was subsequently diagnosed with brain metastases 2) grade 3 fatigue and 3) atrial fibrillation. No patient required more than one dose reduction. PK sampling demonstrated that the prodrug was cleaved efficiently without regard to food. The most common TEAEs (all grades) occurring in > 40% of patients were transaminitis, increased lipase and nausea. DLT occurred in 3 patients: 1) grade 3 anorexia, 2) grade 3 fatigue and, 3) atrial fibrillation. The MTD was determined to be troriluzole 140 mg QAM + 280 mg PO QPM. The response rate was 7 % (1/14); this occurred in a PD-L1 treated patient. The 6-month PFS rate was 21%.[30]

2.5 Ipilimumab and Nivolumab

Combination dual checkpoint inhibitor therapy with ipilimumab and nivolumab is an FDA approved regimen for metastatic melanoma. As compared to PD-1 monotherapy, this combination is associated with a higher response rate. An analysis of long-term overall survival from the Checkmate–067 study demonstrated that there is a 7% survival advantage at the 4-year mark (53% for ipilimumab plus nivolumab vs 46% for nivolumab alone).[31] Immune therapy is used regardless of BRAF mutational status and in BRAF-mutant patients, it is used as a first-line regimen or following BRAF-directed therapy.

The schedule of therapy is 4 cycles of induction where both ipilimumab and nivolumab are given on the same day in 3-week cycles. Following this 12-week induction. Patients are treated with nivolumab monotherapy for up to 2-years. Combination therapy has a higher rate of grade 3 and 4 toxicities (55%) as compared to single agent therapy, leading to treatment discontinuation in 36% of patients.[32] Patients who experience significant side effects during the induction period generally have an interruption in their therapy while they are requiring corticosteroids. Any remaining cycles of combination therapy are omitted, and upon recovery and discontinuation of steroids, the patient is treated with nivolumab monotherapy. If significant side effects occur on monotherapy, therapy is discontinued.

While patients with untreated brain metastases were generally excluded from all of the early studies, a study published of dedicated to melanoma brain metastases, the Checkmate 204 study, was recently published.[3] In neurologically asymptomatic patients with small volume brain metastases and no previous systemic therapy, the combination was shown to be an active regimen with a response rate of 48%.[3] The response rate in symptomatic patients was far lower. The vast majority of patients have concordant responses in their intracranial and extracranial disease. Stereotactic radiotherapy (SRT) of one brain lesion was allowed on study. The response

rate in this study was 48% and the intracranial and the extracranial response rates were largely concordant. The global PFS was 61% at the 6-month mark (Figure 2.6). Notably, the slope of the PFS curve began to flatten out and only decreased by 5% at the 12-month mark, after which time it remained relatively flat and the median PFS was not reached. This suggests that the weeks to months just after the diagnosis of brain metastases is a key period for intervention.

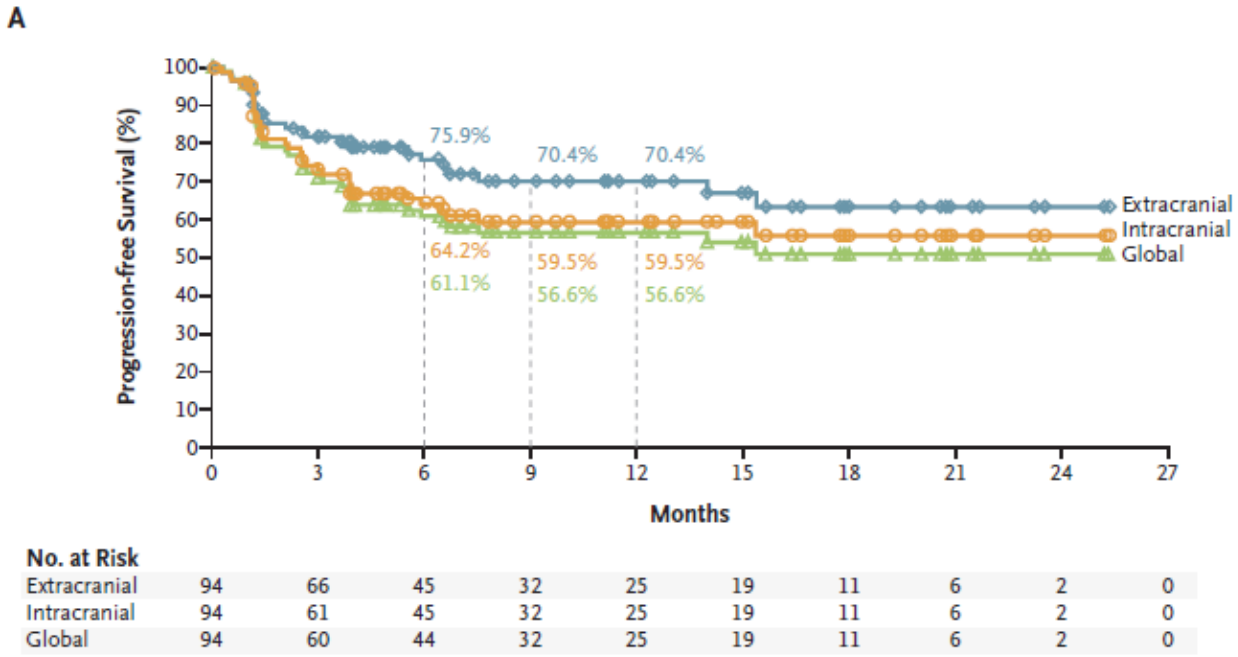


Figure 2.6 PFS curve from the asymptomatic cohort of patients (n=94) in Check-Mate-204.

The study did not identify any new safety signals. Treatment-related adverse event leading to discontinuation of treatment were observed in 27% of subjects, which is not higher than the rate in previous studies.[32] Grade 3-4 treatment-related nervous system adverse events were observed in 8% of patients. These and events included headache (4%), brain edema (1%), intracranial hemorrhage (1%), peripheral motor neuropathy (1%), and syncope (1%).

2.6 Radiation Therapy

Preclinical studies in melanoma and breast cancer cell lines and animal models have demonstrated that riluzole enhances the effectiveness of radiation therapy. To mimic brain metastases observed in melanoma patients, Wall et al developed an intracranial orthotopic transplant model of human melanoma.[33] They injected C8161-luciferase expressing cells intracranially into immunodeficient nude mice and monitored tumor cell establishment using luminescent imaging. Upon initial detection, animals were imaged bi-weekly to determine if riluzole could further sensitize orthotopic tumors to the effects of radiation therapy. The results in the intracranial model show that riluzole and irradiation alone resulted in decreased luminescence compared to vehicle treated alone (Figure 2.7). The combination of riluzole and irradiation resulted in enhanced reduction in tumor cell growth as suggested by luminescent signal detected. Similar outcomes were seen in a glioma model.

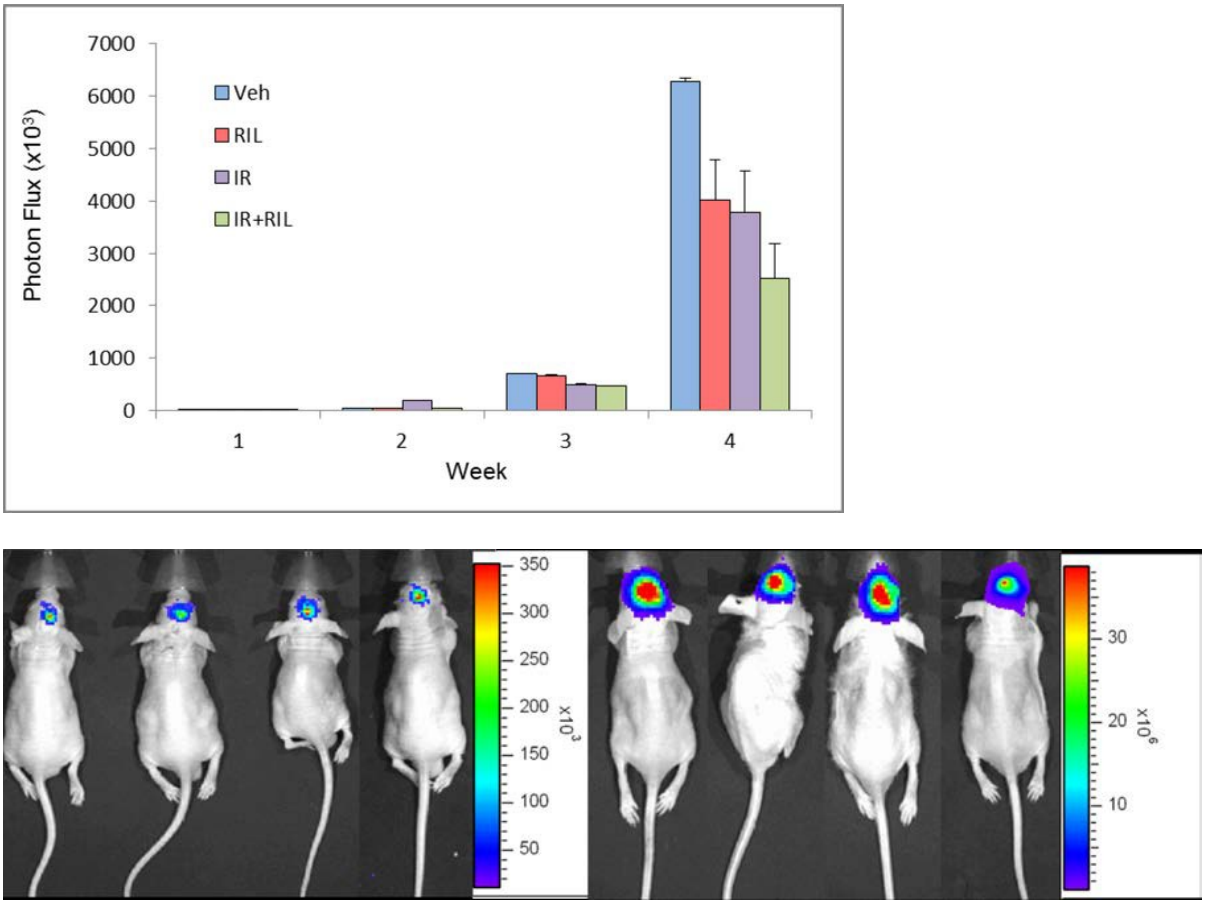


Figure 2.7. Intracranial transplants of human melanoma cells: Left: Week1: C8161-luc+ human melanoma cells were introduced into the brains of nude mice. Once luminescent signal was detected, animals were separated into groups and treated with either DMSO (Veh) or riluzole (Ril, 10 mg/kg), localized ionizing radiation (IR, 4 Gy) or a combination of the two (Ril+IR). Animals were terminated after 4 weeks of treatment. Images of animals showing initials and terminal images **(Left: Week 1**, **Middle: Week 4). Right:** The intensities of the emitted signal were quantified. The data were expressed as photon flux (photons/s/cm2/steradian, where steradian refers to the photons emitted). The background photon flux was subtracted from the signal intensities measured at the same site. Data were normalized to peak signal intensity of each time course.

2.7 Rationale

A key reason for resistance to immune checkpoint blockade is the lack of immune infiltrate in the tumor. In patients with metastatic melanoma, responses to PD-1 inhibitors are more frequent with an inflamed tumor microenvironment, distinguished by high numbers of TILs, high PD-L1 expression, and increased gene expression of the immune-related profile. Thus, adjunct agents that can increase immune infiltration in the tumor microenvironment, or convert a "cold" tumor into a "hot" tumor, are rational combination strategies. In tumors devoid of infiltrating lymphocytes (immune desert), troriluzole treatment may inhibit release of the exosomes and decrease TAMs, allowing lymphocytes into the tumor-stromal interface, thereby, enhancing the antitumor activity of immune checkpoint inhibitors. This hypothesis is supported by preclinical observations using an immunocompetent mouse model of melanoma, which demonstrates that the combination of

troriluzole + PD-1 blocking antibody inhibited the outgrowth of tumors better than either agent alone (Fig. 2.5).

There is an increasingly recognized intersection between antiangiogenic agents and immunotherapy.[25] We have shown that riluzole decreases VEGF levels and increase the immune infiltrate in tumor specimens from melanoma patients.[24] Others have shown that anti-angiogenic therapy can increase the efficacy of checkpoint blockade in uterine cancer and kidney cancer.[27-29] There are numerous ongoing clinical studies in various cancers using a similar rationale, combining immune checkpoint blockade with either an anti-angiogenesis agent (e.g. bevacizumab, ramucurimab), or a macrophage-directed agent (e.g. CSF1 antibody, IPI-549). However, our approach is unique in two ways: 1) that troriluzole is a pro-drug of a well-known entity riluzole that has been FDA-approved for a non-oncology indication for over two decades, and therefore the safety track record is superior, and 2) riluzole crosses the blood-brain barrier and synergizes with radiation therapy, making it well-suited for study in brain metastases.

Despite recent advances, the care of patients with brain metastases due to melanoma remains very challenging. The FDA has called for inclusion of patients with brain metastases in trials of new agents, especially for diseases but commonly involve the CNS, and welcomed applications to expand an indication to include brain metastases.[34]

Taking ipilimumab and nivolumab as the new standard of care for treatment of small asymptomatic melanoma brain metastases, the randomized phase 2 study described herein will attempt to build on the success of this regimen with the addition of troriluzole. The starting dose of troriluzole was determined in the phase 1b study with nivolumab. Patients in both arms may receive brain-directed stereotactic radiotherapy as needed. The rationale to include stereotactic radiosurgery in this trial is that it is standard of care to radiate isolated brain metastases and continue systemic therapy if it is otherwise effective, as was done in the Checkmate 2004 study.[3] As there is preclinical synergy with radiation therapy with troriluzole this is something we will to investigate further in this trial in a subset analysis.

2.8 Correlative Studies Background

The primary goal of the correlative science is to obtain an understanding of the effects of the three-drug regimen vs. the two-drug regimen upon anti-cancer immunological activity. To that end, this study will include the following correlative studies:

- IHC on archival and fresh tumor samples. Tumors from patients who respond favorably to checkpoint blockade therapy showed higher pre-existing numbers of CD8, PD-1, and PD-L1 expressing cells in the tumors and at the invasive tumor margin (tumor-stroma interface) compared to non-responders.[35,36] Response rate are often observed to be approximately 3 times higher in patients with tumors expressing high levels of PD-L1. IHC will be used to determine GRM1 expression, PD-L1 expression, the degree of TILs, and other immune effector cells, and on-treatment biopsies will be examined for change in TILS and decrease in signal transduction in key pathways (e.g. MAPK, Pi3K/AKT) and evidence of apoptosis (cleaved caspases). We hypothesize that nearly 100% of melanomas will have GRM1

overexpression.

- Flow cytometry on PBMCs. Immunophenotypic analysis of immune cells and markers will be assessed by multiparameter flow cytometry. We hypothesize that responders will display more favorable T cell population ratios, markers of activation, and differentiation, and fewer MDSCs.

- Multiplex cytokine analysis on serum and CSF. We hypothesize that responders will demonstrate a decrease in the expression of VEGF, IL-8, CD34, CCL2, and M-CSF.

- Gene expression analysis by Nanostring on blood and tumor samples. We hypothesize that responders will have a higher immune-related gene expression profile. We hypothesize there will be a decrease in oxidative phosphorylation genes post-treatment.

- Tumor genomic profiling. We will determine if activating mutations in B-RAF or N-RAS, or PTEN inactivation (or activating B-RAF mutations and PTEN inactivation together) in the pre-treatment tumor samples correlates with response to therapy.

- Exosome analysis. We will examine exosome contents and hypothesize that post-treatment serum samples will contain fewer suppressive cytokines than pre-treatment samples.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.101 Participants must have histologically or cytologically confirmed melanoma. All melanoma subtypes are included, except for ocular melanoma.

3.102 Participants must have measurable disease in the brain (intraparenchymal brain metastases), defined as at least one lesion that can be accurately measured by MRI in at least one dimension as ≥5 mm and ≤ 3 cm in longest diameter. See Section 11 (Measurement of Effect) for the evaluation of measurable disease. Measurable disease in the extracranial compartment (body) is not required. Measurable lesions may not have received previous treatment with radiation therapy. Prior stereotactic radiation therapy or SRT (e.g. GammaKnife, CyberKnife) is allowed for lesions other than the lesions selected as measurable target lesions. Prior craniotomy with resection of brain metastases is allowed.

3.103 Participants are allowed, but not required to have received prior systemic treatment with anti-PD-1 therapy (e.g. pembrolizumab, or nivolumab) in any setting (neoadjuvant, adjuvant or metastatic). Prior anti-CTLA-4 monotherapy is allowed (e.g. ipilimumab). Prior targeted therapy (e.g.

BRAF inhibitors, MEK inhibitors) is allowed.

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

3.105 ECOG performance status 0 or 1 (see Appendix A).

3.106 Participants must have adequate organ and marrow function as defined below:

- absolute neutrophil count ≥1,000/mcL
- total bilirubin ≤ 1.5 x institutional upper limit of normal (ULN), or in the case of Gilbert's disease ≤ 3x ULN
- AST(SGOT)/ALT(SGPT) ≤3 × institutional ULN

3.107 Human immunodeficiency virus (HIV)-infected participants on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.

3.108 For participants with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

3.109 Participants with a history of hepatitis C virus (HCV) infection must have been treated and cured. For participants with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load.

3.110 Participants with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, participants should be class 2B or better.

3.111 The effects of troriluzole on the developing human fetus are unknown. For this reason and because ipilimumab is a pregnancy category C, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence from heterosexual intercourse) prior to study entry, for the duration of study participation, and 4 months after completion of all study drugs. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of

study participation, and 4 months after completion of all study drugs.

3.112 Ability to swallow pills.

3.113 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.201 Ocular subtype of melanoma.

3.202 Cytologically confirmed leptomeningeal metastases, or convincing imaging evidence of leptomeningeal spread.

3.203 Prior whole brain radiation therapy (WBRT).

3.204 Prior combination therapy with concurrent ipilimumab (3 mg/kg IV) + nivolumab (1 mg/kg IV) in the 24 months prior to the date of registration.

3.205 Participants who have had systemic therapy (immunotherapy, chemotherapy, or targeted therapy), or major surgery within 3 weeks prior to the date of registration. Participants who have had radiotherapy within 10 days prior to the date of registration.

3.206 Participants who require immediate local treatment (surgical resection or radiosurgery) of brain metastases due to neurological symptoms, or brain metastases located in sensitive areas of the brain requiring immediate local treatment.

3.207 Participants who have required systemic steroids to manage neurologic symptoms (seizures, cerebral edema, severe headache, nausea/vomiting, etc.) within 1 week prior to the date of registration.

3.208 Participants who are receiving any other investigational agents for cancer or neurologic disease.

3.209 Extreme claustrophobia that would interfere with performing brain MRIs or severe allergy to gadolinium contrast.

3.210 History of severe or life-threatening allergic reactions attributed to compounds of similar chemical or biologic composition to troriluzole, riluzuole, ipilimumab, or nivolumab.

3.211 Second primary malignancy that is a competing cause of death in the opinion of the treating investigator (prognosis < 6 months).

3.212 Patients with a history of solid organ transplant, or allogeneic bone marrow transplant.

3.213 Active autoimmune disease or any other condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other systemic immunosuppressive medications within 3 weeks of registration.

3.214 History of grade 4 immune related adverse event from prior cancer treatment, (with the exception of asymptomatic elevation of serum amylase or lipase).

3.215 History of immune-related adverse event from prior cancer immunotherapy treatment that has not improved to grade 0-1 (with the exception of patients with ongoing thyroid, adrenal or gonadal insufficiency requiring continued medical treatment, vitiligo, or asymptomatic elevation of serum amylase or lipase).

3.216 Participants receiving any medications or substances that are inhibitors or inducers of the liver enzyme Cytochrome P-450 CYP1A2, including fluvoxamine, cimetidine, amiodarone, efavirenz, fluoroquinolones (including ciprofloxacin and levofloxacin), fluvoxamine, furafylline, interferon, methoxsalen, mibefradil, or ticlopidine. These medications must be discontinued at least 7 days prior to registration.

3.217 Participants with uncontrolled intercurrent illness.

3.218 Participants with psychiatric illness/social situations that would limit compliance with study requirements.

3.219 Pregnant and nursing (breastfeeding) women are excluded from this study because the effects of troriluzole on the developing human fetus are unknown, and because ipilimumab is pregnancy category C.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION AND RANDOMIZATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or

intervention begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

The eligibility checklist and all pages of the consent form will be faxed to the DF/HCC Office of Data Quality (ODQ) at 617-632-2295. The ODQ will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) when applicable, randomize the participant.

Randomization can only occur during ODQ business hours (8:30am – 5pm Eastern Time, Monday through Friday excluding holidays).

An email confirmation of the registration and/or randomization will be sent to the PI, study coordinator(s) from the registering site, treating investigator and registering person immediately following the registration and/or randomization.

As this is a double-blinded study in the randomized portion (Part II), an email confirmation of the treatment assignment (troriluzole vs. placebo) will be sent to the investigational pharmacy team.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Principal Investigator (PI) of the registering site. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at DF/HCC by the Study Coordinator. All sites should contact the Study Coordinator at DFC to verify slot availability: DFCIMelanomaCRCs@partnershealthcare.onmicrosoft.com

The required form, the External Site Subject Registration Form, can be found in Appendix E.

Following registration, participants should begin protocol therapy within 10 days. Issues that would cause treatment delays should be discussed with the Sponsor-Investigator. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the participating site and faxed (Fax # 617-582-8216) or e-mailed (DFCIMelanomaCRCs@partnershealthcare.onmicrosoft.com) to the Study Coordinator at DFCI:

- Copy of brain MRI
- Signed participant consent form
- HIPAA authorization form (if not included in consent form)
- Eligibility Checklist
- External Site Subject Registration Form (Appendix E)

The participating site will then contact the Study Coordinator at DFCI (DFCIMelanomaCRCs@partnershealthcare.onmicrosoft.com) to verify eligibility. The Study Coordinator will follow DF/HCC policy (REGIST-101) and register the participant on the protocol. The Study Coordinator will fax or e-mail the participant study number to the participating site. The Study Coordinator will also contact the participating site and confirm registration.

5. TREATMENT PLAN

5.1 Treatment Regimen

Cycle length is variable during this protocol. During the induction phase, cycles are 3 weeks. During the maintenance phase, cycles are 4 weeks.

5.1.1 Safety Run-In:

The starting dose of the Safety Run-In is outlined below. Up to three cohorts of participants will be treated in a 3+3 design. At the end of each cohort accrual of 3 participants, enrollment will pause for a comprehensive review of the safety data including dose-limiting toxicity (DLT). A starting dose of Troriluzole at 140 mg/280 mg will be used. If there are ≥2 DLTs as defined in Section 6.2, the fallback dose in cohort -1 will be used for three new participants accrued to Cohort -1. If there are < 2 DLTs in the first three participants, an additional three participants will be added to the starting cohort. From this point, if fewer than two patients within a cohort have DLTs then that dose level will be considered the MTD and enrollment to the randomized trial will begin. If two or more patients within a safety cohort have DLTs, then the dose will either be de-escalated, or the trial will stop. A participant will be considered to have completed the DLT period with no dose limiting toxicities only if they have taken ≥ 80% of their assigned Troriluzole doses in the DLT period, which is the first 6 weeks of treatment.

Table 5.11 Cohort de-escalation table during safety run-in:

Dose Level	Troriluzole Dose
Starting cohort	140 mg PO QAM and 280 mg PO QPM
Cohort -1	140 mg PO BID

Cohort -2	140 mg PO daily
If ≥2 DLTs on Cohort -2	Permanently stop the trial

During the induction phase:
Nivolumab (1 mg/kg) followed by ipilimumab (3 mg/kg) will be administered every 3 weeks, with 21 consecutive days defined as a treatment cycle. Troriluzole will be continuously self-administered orally twice a day (at the dose level indicated by participant's cohort).

During the maintenance phase:
Nivolumab (480 mg IV) will be administered every 4 weeks, with 28 consecutive days defined as a treatment cycle. Troriluzole will be continuously self-administered orally twice a day (at the dose level indicated by participant's cohort).

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Table 5.12

Regimen Description – Induction					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Troriluzole	*Take with or without food.*	*Twice daily per cohort assigned dose*	*Oral, 140 mg capsules*	*Days 1-21**	*3 weeks*
Nivolumab		*1 mg/kg over 30 minutes***	*IV*	*Day 1*	
Ipilimumab		*3 mg/kg over 30 minutes***	*IV 0-60 min after completion of nivolumab through separate IV line*	*Day 1*	
The participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle* * Infusion time windows of -5 min/+ 10 min are allowed*					

Regimen Description – Maintenance					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***

Troriluzole or placebo	*Take with or without food.*	*Twice daily per cohort assigned dose*	*Oral, 140 mg capsules*	*Days 1-28**	*4 weeks*
Nivolumab		*480 mg over 30 minutes***	*IV*	*Day 1*	
***The participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle* *** Infusion time windows of -5 min/+ 10 min are allowed*					

5.1.2 Randomized Treatment

During the induction phase:
Nivolumab (1 mg/kg) followed by ipilimumab (3 mg/kg) will be administered every 3 weeks, with 21 consecutive days defined as a treatment cycle. Troriluzole vs. placebo will be continuously self-administered orally twice a day (140 mg PO QAM and 280mg PO QPM**).

During the maintenance phase:
Nivolumab (480 mg IV) will be administered every 4 weeks, with 28 consecutive days defined as a treatment cycle. Troriluzole vs. placebo will be continuously self-administered orally twice a day (140 mg PO QAM and 280mg PO QPM**).

**This is the anticipated MTD from the Safety Run-In. If the Safety Run-In fallback cohorts are needed and the MTD is lowered, this will be amended.

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Table 5.13

Regimen Description – Induction					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Troriluzole or placebo	*Take with or without food.*	*1 cap oral Q AM; 2 caps oral QPM*	*Oral, 140 mg capsules*	*Days 1-21**	*3 weeks*
Nivolumab		*1 mg/kg over 30 minutes***	*IV*	*Day 1*	

Ipilimumab		*3 mg/kg over 30 minutes***	*IV 0-60 min after completion of nivolumab through separate IV line*	*Day 1*	
The participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle* * Infusion time windows of -5 min/+ 10 min are allowed*					

Regimen Description – Maintenance					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Troriluzole or placebo	*Take with or without food.*	*1 cap oral Q AM; 2 caps oral QPM*	*Oral, 140 mg capsules*	*Days 1-28**	*4 weeks*
Nivolumab		*480 mg over 30 min***	*IV*	*Day 1*	
The participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle* * Infusion time windows of -5 min/+ 10 min are allowed*					

5.2 Pre-Treatment Criteria

Safety Run-In and Randomized Treatment:

Cycle 1, Day 1. The subject must meet all eligibility criteria on Day 1. Baseline laboratory and clinical evaluations are to be conducted within 1 week prior to start of protocol therapy. If the participant's condition is deteriorating, evaluations should be repeated within 48 hours prior to initiation of therapy. Complete blood count with differential and comprehensive metabolic panel results should be reviewed prior to treatment. It is recommended that participants receive all indicated routine immunizations prior to the start of treatment (due to possible effect on immunologic correlative science and desire to minimize toxicity during the induction phase).

Subsequent Cycles. Safety assessments, including complete blood count with differential and comprehensive metabolic panel results must be performed prior to administration of any study agent, excluding oral therapy, which is continuous. Study assessments and agents should be administered within + 7 days of the protocol-specified date for Days 1-309, and within + 14 days of the protocol-specified date for Days 337-673.

5.3 Agent Administration

Safety Run-In and Randomized Treatment:

Administration and Dosing. As nivolumab and ipilimumab are FDA approved for metastatic melanoma, administration will follow institutional standard/package insert. The IV agents are not vesicants or irritants. No observation period is necessary. Doses of nivolumab and ipilimumab are weight-based during the induction phase. It is acceptable to use baseline weight for all induction cycles. In the instance that the institutional policy is more strict, institutional policy should be followed. Refer to table in Section 5.1. Details are provided below for reference.

> During the induction phase:
> Nivolumab (1 mg/kg) followed by ipilimumab (3 mg/kg) 0-60 minutes later will be administered every 3 weeks, with 21 consecutive days defined as a treatment cycle. Troriluzole vs. placebo will be continuously self-administered orally twice a day (140 mg PO QAM and 280mg PO QPM; or twice daily per cohort assigned dose if in Safety Run-In). Due to toxicity, not all patients are expected to complete all 4 cycles of the induction. See section 6 for dosing modifications/omissions.
>
> During the maintenance phase:
> Nivolumab (480 mg IV) will be administered every 4 weeks, with 28 consecutive days defined as a treatment cycle. Troriluzole vs. placebo will be continuously self-administered orally twice a day (140 mg PO QAM and 280mg PO QPM; or twice daily per cohort assigned dose if in Safety Run-In).
>
> Oral agents, troriluzole vs. placebo. Troriluzole or placebo in a blinded fashion will be self-administered orally with BID dosing Q 12 hours (± 2 hours). On a continuous basis throughout the protocol. Capsules can be taken with or without food; if a patient experiences nausea, taking the capsule with food is recommended. Capsules cannot be crushed, chewed, or dissolved. If a dose has been missed or it is vomited up, it should be skipped. Patients may choose to use an antiemetic prior to taking troriluzole if needed.

Order of administration: On Cycle 1 Day 1, the nivolumab will be administered first, followed by the ipilimumab, and in the evening, the first dose of troriluzole or placebo will be self-administered at home. For subsequent cycles, troriluzole or placebo will be self-administered in the morning before coming to the outpatient infusion center, followed by the infusion(s), and then troriluzole will be self-administered in the evening.

Management of infusion reactions: refer to institutional policy; see section 6.4 for additional guidance. Steroids should be avoided as a routine premedication due to possible interference with the efficacy of the study agents. Pre-medications are not required but are allowed for patients with previous infusion reaction.

Treatment Period.

> The treatment period of the induction phase is Day 1-84 (12 weeks).
>
> The treatment period of the maintenance phase is from Day 85 to 337 (36 weeks). Day

337 is the last protocol-directed infusion of nivolumab (see below) and the last dose of troriluzole.

Adherence/Compliance. At the visit, each subject will be given a bottle containing capsules of study drug. The participant will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each cycle. Unused study medication will be returned to the research pharmacy. At the end of each cycle of treatment, the actual amount of unused drug will be compared to the amount taken as recorded on the subject's medication diary. The desired level of compliance is >75%. If a subject is below that threshold, the treating investigator should assess barriers to compliance and address any correctable barriers (e.g. antiemetic therapy). Patients who cannot maintain this level of compliance may be removed from the study at the discretion of the treating investigator.

5.4 End of Treatment

Safety Run-In and Randomized Treatment:

The End of Treatment (EOT) visit, Day 337 (or sooner if the patient discontinues active treatment) is the last dose of nivolumab and the last dose of Troriluzole vs. placebo. EOT assessments are listed in Section 10 (Study Calendar).

Participants removed from active protocol therapy for unacceptable adverse event(s) will receive management and supportive care for the adverse event until resolution or stabilization of the adverse event, per the treating investigator. During this time, participants must present for in-person visits at least every 12 weeks ± 1 week.

Following EOT, participants may continue on nivolumab therapy indefinitely, as it is FDA approved without a maximum duration. Continuation of nivolumab is not required for this protocol. Patients who have completed the last dose of study medication (Day 337) should discuss their individual treatment plans with their treating physician. See Section 5.9 for protocol-required follow up.

5.5 Concomitant Medication and Supportive Care Guidelines

Safety Run-In and Randomized Treatment:

Acceptable Concomitant Medications. All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids.

Selected acceptable medications include:

- Antiepileptic (anti-seizure, anticonvulsant) medications. *Exception*: Carbamazepine, which is a CYP1A2 inducer, is prohibited.

- Erythropoietin
- Hormone replacement therapy
- Immunizations. Routine vaccinations are permitted but discouraged during Days 1-84 when the most toxicity is expected. If the treating investigator feels that a vaccination is unsafe to delay (e.g. seasonal influenza immunization), the patient may receive the vaccination during Days 1-84. Vaccinations received during the study period should be documented as a concomitant medication.
- Anticoagulation. Prophylactic or treatment-dose anticoagulation is permitted when indicated. ASCO has recently published new a guideline on venous thromboembolism prophylaxis and treatment in patients with cancer. Intracranial or CNS bleeding within the past 4 weeks is considered a relative contraindication to therapeutic anticoagulation.[37]
- Steroids (dexamethasone, prednisone, etc.) – allowable indications
    - During study participation, neurologic symptoms may arise or worsen. Steroids may be used to control neurologic symptoms. Steroid treatment ≤ 16 mg dexamethasone PO daily tapered in ≤ 4 weeks is allowed for the treatment of brain edema (single episode). If a second episode of brain edema requires steroid treatment, the Sponsor-Investigator must be consulted.
    - Systemic steroids may be used for treatment of irAEs.
    - A brief course (< 7 days) of corticosteroids for prophylaxis of side effects from SRT is permitted.
    - A brief course (< 7 days) of corticosteroids for prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by contact allergen) is permitted.
    - Intranasal, intra-articular, and inhaled steroids are acceptable for any indication.
    - NOTE: Patients who receive systemic corticosteroids for any reason (including toxicity or management of symptoms brain metastases) must be off corticosteroids or have tapered down to an equivalent dose of prednisone 10 mg/day (dexamethasone ≤1.5 mg PO daily) or less to continue immune checkpoint therapy.

Prohibited Medications

- Systemic steroids for indications other than the indications above
- No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.
- Prophylactic use of colony stimulating factors (CSFs) are not permitted. They may be needed for supportive care and should be discontinued when the event resolves/improves. If needed longer than 4 weeks, the subject must discontinue study treatment.
- IVIG

5.6 Concomitant Brain-Directed Stereotactic Radiation therapy during the study

Safety Run-In and Randomized Treatment:

Permitted Therapy: Brain-directed Stereotactic Radiotherapy (SRT), also called stereotactic radiosurgery (SRS). If a subject demonstrates clinical benefit, but disease progression in ≤ 4 intracranial metastases occurs, the investigators may prescribe SRT (single episode), and 2

scenarios are permitted as detailed below.

- If the patient is clinically stable, progression should be confirmed by follow-up imaging after 4 weeks. If progression is confirmed, the patient may undergo SRT treatment (single episode in 1 – 5* fractions) for ≤ 4 lesions and may receive ≤ 16 mg dexamethasone PO daily tapered in ≤ 4 weeks. Treatment with the study drugs can be resumed after taper completion as long as the patient does not demonstrate criteria for discontinuation.

  Note: Treatment in 5 fractions is generally indicated for lesions in critical anatomical locations, such as in the brainstem or abutting the optic chiasm.
- If the patient is symptomatic as a result of the disease progression, and clinical assessment indicates a requirement for SRT without the delay imposed by the 4 week confirmatory scan, the patient may undergo SRT and may receive ≤ 16 mg dexamethasone PO daily tapered in ≤ 4 weeks. Treatment with the study drugs can be resumed after the completion of the taper as long as the patient does not demonstrate criteria for discontinuation.

As a general guide, efforts should be made to avoid radiotherapy within 2 weeks after a treatment of either nivolumab + ipilimumab combination therapy or nivolumab monotherapy and avoid treatment resumption until at least 1 treatment-cycle length after radiotherapy.

These guidelines are adapted from a previous protocol (Tawbi, *et al*) that allowed SRT for ≤ 3 lesions,[3] and adapted as per Aoyama, *et al* to allow SRT to up to 4 lesions.[6] The ability to use 5 fractions for lesions in anatomically critical areas as described above is a second adaptation.

Administration of SRT and size of irradiated target lesions will be recorded on case report forms. The data from subjects who receive on-study SRT will be analyzed as a "radiation salvage" subgroup.

Permitted Therapy: Palliative Radiotherapy to a non-target lesion. Radiation therapy may be performed to palliate an extracranial tumor as long as it is not a target lesion for tumor assessment.

5.7 Concomitant Surgery therapy during the study

Safety Run-In and Randomized Treatment:

If a subject has disease progression in the brain and craniotomy or other skull surgery is recommended per standard of care, a single surgical intervention is permitted with the approval of the Sponsor-Investigator. To remain on-study, the subject must have remaining measurable disease in the brain.

Surgery for non-cancer indications may be allowed. The case should be discussed with the Sponsor-Investigator.

5.8 Criteria for Taking a Participant Off Protocol Therapy

Safety Run-In and Randomized Treatment:

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until Day 337 or until one of the following criteria applies:

- Disease progression, with the following exceptions:
  - To allow for the possibility of pseudoprogression, investigators may continue to treat a patient beyond progression until the next assessment if the investigator believes the patient is clinically benefitting (must document in progress note). If PD is confirmed on a subsequent scan, therapy must be discontinued.
  - Investigators may continue to treat a patient beyond progression if the progressing lesion(s) can be addressed with brain-directed SRT (See Section 5.6). *NOTE*: To continue on study after SRT, the subject must have at least 1 non-irradiated target lesion and/or non-target lesion in the brain or body.

- Intercurrent illness that prevents further administration of treatment

- Unacceptable adverse event(s)

- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements

- Participant decides to withdraw from the protocol therapy

- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the participant's status must be updated in OnCore in accordance with REGIST-OP-1.

5.9 Duration of Follow Up

Safety Run-In and Randomized Treatment:

Participants will be followed with in-person visits on Days 421, 505, and 589, or until death, whichever occurs first. If they are receiving additional nivolumab, this should be documented in the progress notes and reported in the case report forms. Treatment-emergent AEs should continue to be monitored during the follow-up period (until resolution). New AEs during follow up that are not related to treatment should not be recorded.

Tumor assessments will be performed as per standard of care during Days 421-589 and will be captured on CRFs.

Day 673 is the last required in-person visit. Subjects will continue to be contacted (or their charts will be accessed) for information on subsequent therapy and vital status for a total of 3 years. The study team will contact participants by phone every 12 weeks (+/- 14 days) to assess survival.

5.10 Criteria for Taking a Participant Off Study

Safety Run-In and Randomized Treatment:

Participants will be removed from study (including study follow-up) when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure the participant's status is updated in OnCore in accordance with REGIST-OP-1.

5.11 Requests for Unblinding

Only applicable for Randomized Treatment:

Requests for unblinding may be granted under the following circumstances

1. Emergency unblinding- If there is a serious adverse event or other severe illness and the study drug is suspected to be a factor, a request for unblinding may be submitted to the study team/pharmacy. It is preferable that the decision to unblind is discussed with the Principal Investigator, but the treating investigator holds the final authority to request emergency unblinding to protect the safety of participants. The study team will contact the pharmacy to facilitate unblinding.
    a. Contact #1 for Emergency Unblinding:
    David Husselbee – Research Manager
    Telephone: 617-632-6704 // Fax: 617-582-8216
    David_Husselbee@DFCI.HARVARD.EDU
    b. Contact #3 for Emergency Unblinding:
    DFCI CRC General Inbox
    DFCIMelanomaCRCs@partnershealthcare.onmicrosoft.com

24-hour contact for emergency unblinding:
Ann W. Silk, MD
Pager: Call (617) 632-3352 for beeper # 41109

2. Elective unblinding – Patients or their treating investigators who would like to know if they have received troriluzole or not may request unblinding from the study team on Day 365. Unblinding will be facilitated by the study team members:
    a. John Collantes – Study Coordinator
    Telephone: 617-632-3469 // Fax: 617-582-8216
    JohnV_Collantes@dfci.harvard.edu
    b. David Husselbee – Research Manager
    Telephone: 617-632-6704 // Fax: 617-582-8216
    David_Husselbee@DFCI.HARVARD.EDU

3. Final Unblinding

The official, final database will not be unblinded until medical/scientific review has been performed, protocol deviations have been identified, and data have been declared final and complete.

The final unblinding will include distribution of a subject letter to participants and subject treatment information to study investigators. Final unblinding will occur after enrollment has been completed and the Sponsor-Investigator has determined that study data are final and complete.

A planned futility interim analysis will be conducted during the randomized trial. Blinding to treatment assignment will be maintained at all investigational sites at the time of the futility analysis. The unblinded results of interim analyses will not be shared with the investigators prior to the completion of the study. Subject-level unblinding will be restricted to an unblinded statistician and scientific programmer performing the futility analysis.

The Sponsor-Investigator will serve as the primary reviewer of the results of the interim analysis and will follow the protocol-defined guidance in making a decision on discontinuation of the study or modification.

Subject Letter.
At the close of the trial after unblinding, a letter is to be sent by the investigator to those subjects who received placebos or investigational product to provide information on the trial and include the following advice:

> *"You have participated in a trial conducted by Dana-Farber Cancer Institute under the sponsorship of Biohaven Pharmaceuticals. This is to advise you that you were among those who received a look-alike capsule created by the Sponsor to resemble the study drug troriluzole as much as possible. You did not receive the active drug troriluzole as manufactured by Biohaven Pharmaceuticals."*

Or

"You have participated in a trial conducted by Dana-Farber Cancer Institute under the sponsorship of Biohaven Pharmaceuticals. This trial included participants that received active drug and participants that received a look-alike capsule created by the Sponsor to resemble the study drug and act as a placebo. This is to advise you that you were among those who received the active drug troriluzole as manufactured by Biohaven Pharmaceuticals."

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 General considerations

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays) and do not constitute a protocol violation. Subjects should be placed back on study therapy within 4 weeks of the missed dose, unless otherwise discussed with the study PI. The reason for interruption should be documented in the patient's study record. The maximum dose interruption of the checkpoint inhibitors (no administrations) for any reason is 84 calendar days (12 weeks). The maximum dose interruption of the Troriluzole for any reason is 42 calendar days (6 weeks). Missed doses are not to be made up; efforts will be made to get the patient back on the original schedule so that assessments are uniform across subjects.

For AEs unrelated to study drug(s), therapy may be held/resumed at the discretion of the treating investigator. Treatment-emergent AEs should continue to be monitored during the follow-up period (until resolution). New AEs during follow up that are not related to treatment should not be recorded

6.2 Dose Limiting Toxicities

For patients participating in the safety run-in, the following definition of dose-limiting toxicities (DLT) will be used:

The occurrence of any AE that meets all three of the following criteria:

1. Grade ≥3 toxicity **or** select grade 2 neurologic toxicity lasting 4 days or more, with or without medical intervention:
    - Ataxia
    - Central nervous system necrosis (use this term for radiation necrosis)

- Cognitive disturbance
- Concentration impairment
- Depressed level of consciousness
- Dizziness
- Encephalopathy
- Guillain-Barre syndrome
- Headache
- Intracranial hemorrhage
- Ischemia cerebrovascular
- Leukoencephalopathy
- Memory impairment
- Presyncope
- Reversible posterior leukoencephalopathy syndrome (PRES)
- Stroke
- Somnolence
  Confusion (categorized under Psychiatric disorders)

2. Occurred during the DLT evaluation period (i.e., occurred during the first 6 weeks of therapy), and
3. Judged by the treating investigator to be possibly, probably or definitely related to troriluzole. Grade ≥3 toxicities that are related to ipilimumab and/or nivolumab but are unlikely related or not related to troriluzole are not considered DLTs. See Section 6.7 for list of Overlapping toxicities ("shared AEs"). Troriluzole therapy will be continued if there is an interruption in checkpoint therapy due to AE, and the AE is unlikely related or unrelated to troriluzole. Troriluzole and corticosteroids can be taken concurrently.

6.3 Troriluzole

General

Troriluzole therapy will be continued if there is an interruption in checkpoint therapy due to AE, and the AE is unlikely related or unrelated to troriluzole. Troriluzole and corticosteroids can be taken concurrently. Nivolumab and/or ipilimumab may still be given if Troriluzole is held or discontinued, if AE is unlikely related or unrelated to the checkpoint inhibitor treatments.

The maximum amount of time that troriluzole can be held continuously is 6 weeks. If the patient cannot resume troriluzole within 6 weeks, the patient will be removed from the study.

If a participant experiences multiple adverse events and there are conflicting recommendations, the investigator should use the recommended dose adjustment that reduces the dose to the lowest level.

Holding criteria

Troriluzole vs. placebo will be omitted and dose reduced for related AEs as follows.

Grade 1
For grade 1 events, dose holding is not required. If the grade 1 toxicity is intolerable to the patient, the drug may be held for 1 week at a time without lowering the dose. If drug is held for >1 week at a time, drug will be dose-reduced **one** level upon resumption. The intolerable nature of the AE must be documented as the reason for holding drug.

Grade 2
For grade 2 events, dose holding is required. When troriluzole is interrupted for an AE, the toxicity should be continually reassessed until it improves to Grade ≤1, on approximately a weekly basis, which can be done remotely by telephone and/or lab assessment. If patient is able to resume drug ≤1 week, drug will be continued at the same dose, or, at the discretion of the treating investigator, it may be dose-reduced. If drug is held for >1 week at a time, it should be dose-reduced one level upon resumption. If grade 2 AE meets criteria for DLT (see section 6.2), troriluzole should be dose-reduced one level upon resumption.

Grade 3
For grade 3 events, dose holding is required. When troriluzole is interrupted for an AE, the toxicity should be continually reassessed until it improves to Grade ≤1, on approximately a weekly basis, which can be done remotely by telephone and/or lab assessment. If the treating investigator believes permanent discontinuation is in the best interest of the patient (e.g. anaphylaxis), Troriluzole may be permanently discontinued after a discussion between the treating investigator and principal investigator. If the patient is able to resume drug, drug will be dose-reduced **one level**.

Grade 4
For grade 4 events, dose holding is required. When troriluzole is interrupted for an AE, the toxicity should be continually reassessed until it improves to Grade ≤1, on approximately a weekly basis, which can be done remotely by telephone and/or lab assessment. If the treating investigator believes permanent discontinuation is in the best interest of the patient (e.g. anaphylaxis), troriluzole may be permanently discontinued after a discussion between the treating investigator and principal investigator. If the patient is able to resume drug, drug will be dose-reduced **two levels**.

Troriluzole vs. Placebo Dose Reductions

No specific management is indicated when troriluzole vs. placebo is held, other than supportive care. When therapy resumes, the dose must be reduced by one level per the table below. The maximum number of dose reductions is 2 as shown in the following de-escalation table.

Dose reductions for troriluzole

Dose Level	Troriluzole vs. Placebo Dose
0	140 mg PO QAM and 280 mg PO QPM
-1	140 mg PO BID
-2	140 mg PO daily

Intra-participant dose reescalation. Intra-participant dose reescalation is allowed. At the discretion of the treating physician, a patient who has previously been dose-reduced may re-escalate up to the starting dose. No patient should take more than the starting dose (Cohort 0). For example, if a AE improves with additional supportive measures (antiemetics, etc), or an alternate cause of the AE is identified, the physician and patient may opt to re-escalate.

6.4 Ipilimumab and Nivolumab

Dose modifications for nivolumab in combination with ipilimumab by adverse event type are detailed in Table 6.1. No dose reductions are allowed. General considerations for holding the dose(s) are as follows.

For the initial induction period, patients with Grade 2 or 3 events requiring discontinuation of treatment with the combination may consider continuing treatment with single agent nivolumab when the event resolves to grade 0-1 (or baseline, when baseline grade is >1). If induction cycles (Ipilimumab + Nivolumab) are held, they will be omitted, not delayed.

Nivolumab monotherapy may be continued at treating investigator discretion if there is evidence of clinical benefit. In this event, patient will continue nivolumab until AE is resolved and ipilimumab may be re-started or if ipilimumab is permanently discontinued (in congruence with guidelines in table below), then patient will continue nivolumab alone until cycles are completed. At completion of 4 dose of combination IPI + NIVO, patient may continue on nivolumab alone, as per protocol. If ipilimumab is permanently discontinued prior to receipt of 4 doses, nivolumab dosing should remain at the induction dosing (refer to Section 5) until completion of induction, and should then switch to maintenance dosing (refer to Section 5).

> Example: Following IPI + NIVO treatment on Day 22, there is a grade 3 AE and Day 43 is held. The patient is treated with steroids and the grade 3 AE resolves quickly. The patient has been tapered down to a steroid dose of prednisone 5 mg po daily by Day 64 and the investigator wishes to continue protocol therapy with nivolumab monotherapy. The patient will receive nivolumab (induction dosing) on Day 64, and then continue as per the Study Calendar.

In addition to the AEs identified in the table below, ipilimumab and nivolumab dose should be delayed for any AE, laboratory abnormality or inter-current illness which, in the judgment of the treating investigator, warrants delaying the dose of study medication.

NOTE: Patients who receive systemic corticosteroids for any reason (including toxicity or management of symptoms brain metastases) must be off corticosteroids or have tapered down to an equivalent dose of prednisone 10 mg/day (dexamethasone ≤1.5 mg PO daily) or less to continue immune checkpoint therapy.

Maximum delay: The maximum amount of time that checkpoint inhibitor(s) can be held continuously is 12 weeks. If the patient cannot resume therapy within 12 weeks, the patient will

be removed from treatment with checkpoint inhibitor.

Patients requiring a delay of > 12 weeks due to ongoing immune-related toxicity or inability to taper prednisone to ≤10 mg PO daily (dexamethasone ≤1.5 mg PO daily) must go off checkpoint inhibitor therapy entirely.

Table 6.1 Dose Modification for Related Adverse Events

Treatment-related Adverse Event	Grade of Event	*Nivolumab or Nivolumab/Ipilimumab*
Nausea	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment (exceptions as noted below) [1]
	Grade 4	Off nivolumab and/or ipilimumab treatment
Recommended management: antiemetics.		
Diarrhea (immune-related enterocolitis)	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline
	Grade 3	Off nivolumab and/or ipilimumab treatment (exceptions as noted below) [1]
	Grade 4	Off nivolumab and/or ipilimumab treatment
Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used. Please evaluate pituitary function prior to starting steroids if possible without compromising acute care. Evaluation for all patients for additional causes includes *C. diff*, acute and self-limited infectious and foodborne illness, ischemic bowel, diverticulitis, and IBD.		
Vomiting	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment (exceptions as noted below) [1]
	Grade 4	Off nivolumab and/or ipilimumab treatment
Recommended management: antiemetics.		
Other GI	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment (exceptions as noted below[1])
	Grade 4	Off nivolumab and/or ipilimumab treatment
Patients with Grade 2 or 3 N-V should be evaluated for upper GI inflammation and other immune related events.		
Neurologic	≤ Grade 1	No dose modification

Treatment-related Adverse Event	Grade of Event	***Nivolumab or Nivolumab/Ipilimumab***
events (new, motor, sensory, encephalitis, or intracranial hemorrhage)*	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment (exceptions as noted below) [1]
	Grade 4	Off nivolumab and/or ipilimumab treatment
* Intralesional bleeding events are common in melanoma. Patients with grade 2 intracranial hemorrhage ("moderate symptoms; intervention indicated") may be treated with antiepileptic medication and/or corticosteroids as indicated. Treatment may be held until symptoms improve to grade 1 (asymptomatic). For extralesional bleeding events, a repeat brain MRI may be indicated prior to resuming treatment, at the discretion of the treating investigator.		
Fatigue	≤ Grade 1	No dose modification
	Grade 2	No dose modification
	≥Grade 3	Off nivolumab and/or ipilimumab treatment (exceptions as noted below) [1] – if fatigue is found related to endocrinopathy and clinical symptoms are managed with hormone replacement, patient resume therapy
	Grade 4	Off nivolumab and/or ipilimumab treatment
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, and hepatic, or muscle (CPK) inflammation		
Fever	≤ Grade 1	Evaluate and continue
	Grade 2	Hold until ≤ Grade 1
	Grade 3	Hold until ≤ Grade 1
	Grade 4	Off nivolumab and/or ipilimumab treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several calendar days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever		
Skin drug-related AE	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment (exceptions as noted below) [1]
	Grade 4	Off nivolumab and/or ipilimumab treatment
Patients with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphagoid. Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.		

Treatment-related Adverse Event	Grade of Event	*Nivolumab or Nivolumab/Ipilimumab*
Pneumonitis, broncho-spasm, pulmonary toxicity or interstitial lung disease	≤ Grade 1	No dose modification.
	Grade 2	Hold dose pending evaluation. Consider pulmonary and/or ID consultation. Resume if an alternative diagnosis is rendered.
	Grade 3	Off nivolumab and/or ipilimumab treatment
	Grade 4	Off nivolumab and/or ipilimumab treatment
	Above does not include infusion reactions.	
Distinguishing inflammatory pneumonitis is often a diagnosis of exclusion for patients who do not respond to antibiotics and have no causal organism identified including influenza. Most patients with respiratory failure or hypoxia will be treated with steroids. Bronchoscopy may be required and analysis of lavage fluid for lymphocytic predominance may be helpful. Patients with new lung nodules should be evaluated for sarcoid like granuloma. Please consider recommending seasonal influenza killed vaccine for all patients.		
Thrombocyto-penia	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment
	Grade 4	Off nivolumab and/or ipilimumab treatment
Neutropenia	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment
	Grade 4	Off nivolumab and/or ipilimumab treatment
Renal	≤ Grade 1	*No dose modification*
	Grade 2	Hold until ≤ Grade 1 (or baseline)
	Grade 3	Off nivolumab and/or ipilimumab treatment
	Grade 4	Off nivolumab and/or ipilimumab treatment
Endocrine Hypophysitis Adrenal Insufficiency	≤ Grade 1	No dose modification
	Grade 2	Hold until patients are on a stable replacement hormone regimen. If treated with steroids patients must be stable off steroids for two weeks.
	Grade 3	Hold until patients are on a stable replacement hormone regimen. If treated with steroids patients must be stable off steroids for two weeks.
	Grade 4	Off nivolumab and/or ipilimumab treatment

Treatment-related Adverse Event	Grade of Event	*Nivolumab or Nivolumab/Ipilimumab*
Note all patients with symptomatic pituitary enlargement, exclusive of hormone deficiency, but including severe headache or enlarged pituitary on MRI should be considered Grade 3 events. Isolated thyroid or testosterone deficiency may be treated as Grade 2 if there are no other associated deficiencies and adrenal function is monitored. Please evaluate pituitary function before beginning steroid therapy or replacement therapy of any kind. Note patients with thyroiditis may be retreated on replacement therapy. Patients must be evaluated to rule out pituitary disease prior to initiating thyroid replacement.		
Abnormal liver function (AST/ALT, Total bilirubin, immune-related hepatitis)	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment
	Grade 4	Off nivolumab and/or ipilimumab treatment
Continued treatment of active immune mediated hepatitis may exacerbate ongoing inflammation. Holding drug to evaluate LFT changes and early treatment are recommended. LFT changes may occur during steroid tapers from other events and may occur together with other GI events including cholecystitis/pancreatitis.		
Amylase or lipase, associated with GI symptoms	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Hold until baseline and asymptomatic. Patients who develop symptomatic pancreatitis or DM must be taken off treatment
	Grade 4	Off nivolumab and/or ipilimumab treatment
Patients may develop symptomatic and radiologic evidence of pancreatitis as well as DM and DKA. Lipase elevation may occur during the period of steroid withdrawal and with other immune mediated events or associated with colitis, hepatitis, and patients who have asymptomatic lipase elevation typically have self-limited course and may be retreated.		
Any other laboratory abnormality (except AST/ALT, Total bilirubin, thrombocytopenia, neutropenia, lymphopenia)	≤ Grade 1	No change.
	Grade 2	No change.
	Grade 3	Off nivolumab and/or ipilimumab treatment unless is unrelated to underlying organ pathology and can be managed with electrolyte replacement, hormone replacement, insulin, or no therapy
	Grade 4*	Off nivolumab and/or ipilimumab treatment unless is unrelated to underlying organ pathology and can be managed with electrolyte replacement, hormone replacement, insulin, or no therapy

Treatment-related Adverse Event	Grade of Event	*Nivolumab or Nivolumab/Ipilimumab*
	* Grade 4 lymphopenia or leukopenia or does not require drug discontinuation. * Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset do not require drug discontinuation. * Resolved, well-controlled, or asymptomatic hypothyroidism does not require approval by the Sponsor-Investigator prior to restarting therapy.	
Cardiac*	≤ Grade 1	Hold dose pending evaluation and observation.** Evaluate for signs and symptoms of CHF, ischemia, arrhythmia or myositis. Obtain history EKG, CK (for concomitant myositis), CK-MB. Repeat troponin, CK and EKG 2-3 calendar days. If troponin and labs normalize may resume therapy. If labs worsen or symptoms develop then treat as below. Hold pending evaluation.
	Grade ≥2 with suspected myocarditis	Hold dose.** Admit to hospital. Cardiology consult. Rule out MI and other causes of cardiac disease. Cardiac Monitoring. Cardiac Echo. Consider cardiac MRI and cardiac biopsy. Initiate high dose methylprednisolone. If no improvement within 24 hours, add either infliximab, ATG or tacrolimus. Resume therapy if there is a return to baseline and myocarditis is excluded or considered unlikely.
	Grade ≥2 with confirmed myocarditis	Off nivolumab and/or ipilimumab treatment. Admit to CCU (consider transfer to nearest Cardiac Transplant Unit). Treat as above. Consider high dose methylprednisolone. Add ATG or tacrolimus if no improvement. Off treatment.
**Including CHF, LV systolic dysfunction, Myocarditis, CPK, and troponin* ***Patients with evidence of myositis without myocarditis may be treated according as "other event"* Note: The optimal treatment regimen for immune mediated myocarditis has not been established. Since this toxicity has caused patient deaths, an aggressive approach is recommended.		
Infusion	≤ Grade 1	*No dose modification*

Treatment-related Adverse Event	Grade of Event	*Nivolumab or Nivolumab/Ipilimumab*
Reaction	Grade 2	Interrupt study drug. Upon recovery from symptoms, resume study drug at one half the initial infusion rate, then increase incrementally to the initial infusion rate
	Grade 3	Off nivolumab and/or ipilimumab treatment
	Grade 4	Off nivolumab and/or ipilimumab treatment
Patients with fever should be evaluated as clinically appropriate. Patients may experience isolated fever during infusion reactions or up to several calendar days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever		
All other events	≤ Grade 1	No dose modification
	Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below) [1]
	Grade 3	Off nivolumab and/or ipilimumab treatment
	Grade 4	Off nivolumab and/or ipilimumab treatment

Table 6.1 Footnotes

[1]Recommended management: As clinically indicated

Exceptions:

- 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 must go off protocol treatment
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the treating investigator, presents a substantial clinical risk to the patient with continued study drug dosing must go off protocol treatment.
- Any Grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality, that can be managed independently from underlying organ pathology with electrolyte replacement, hormone replacement, insulin or that does not require treatment does not require discontinuation.
- See Section 6.6 for treatment management

For additional management recommendations and considerations, see the ASCO Clinical Practice Guideline Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy.[38]

6.5 Treatment of Infusion Reactions Associated with Ipilimumab and/or Nivolumab

Since ipilimumab contains only human protein sequences, and since nivolumab is a fully human monoclonal antibody, it is less likely that any allergic reaction will be seen in patients receiving these agents. However, it is possible that infusion of ipilimumab or nivolumab will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypotension, hypertension, bronchospasm, or other symptoms. No prophylactic pre-medication

should be given unless indicated by previous experience in an individual patient. Reactions should be treated based upon the following recommendations.

- For mild symptoms (e.g., localized cutaneous reactions such as mild pruritus, flushing, rash):
    - Decrease the rate of infusion until recovery from symptoms, remain at bedside and monitor patient.
    - Complete the infusion at the initial planned rate.
    - Diphenhydramine 50 mg IV may be administered at the discretion of the treating physician and patients may receive additional doses with close monitoring.
    - Premedication with diphenhydramine may be given at the discretion of the investigator for subsequent doses.

- For moderate symptoms (any symptom not listed above [mild symptoms] or below [severe symptoms] such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP >80 mmHg):
    - Interrupt infusion of offending agent.
    - Administer diphenhydramine 50 mg IV.
    - Monitor patient closely until resolution of symptoms.
    - Corticosteroids may abrogate any beneficial immunologic effect, but may be administered at the discretion of the treating physician.
    - Resume infusion after recovery of symptoms.
    - At the discretion of the treating physician, infusion may be resumed at one half the initial infusion rate, then increased incrementally to the initial infusion rate.
    - If symptoms develop after resumption of the infusion, the infusion should be discontinued and no additional ipilimumab or nivolumab should be administered that day.
    - The next dose will be administered at its next scheduled time and may be given with pre-medication (diphenhydramine and acetaminophen) and careful monitoring, following the same treatment guidelines outlined above.
    - At the discretion of the treating physician additional oral or IV antihistamine may be administered prior to dosing.

- For severe symptoms (e.g., any reaction such as bronchospasm, generalized urticaria, systolic blood pressure < 80 mm Hg, or angioedema):
    - Immediately discontinue infusion and disconnect infusion tubing from the patient.
    - Consider bronchodilators, epinephrine 1 mg IV or subcutaneously, and/or diphenhydramine 50 mg IV, with solumedrol 100 mg IV, as needed.
    - Patients should be monitored until the investigator is comfortable that the symptoms will not recur.
    - No further ipilimumab (if related to ipilimumab) or nivolumab (if related to nivolumab) will be administered.

6.5 Treatment of Ipilimumab-Related Isolated Drug Fever

In the event of isolated drug fever, the investigator must use clinical judgment to determine if the

fever is related to the ipilimumab or to an infectious etiology. If a patient experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (investigator discretion) should be instituted and a repeated antipyretic dose at 6 and 12 hours after ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses. If a patient experiences recurrent isolated drug fever following premedication and post dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the investigator should assess the patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further ipilimumab.

6.6 Monitoring and Management of Immune-mediated Adverse Reactions

Immune-mediated Enterocolitis

The clinical presentation of GI immune-related AEs included diarrhea, increase in the frequency of bowel movements, abdominal pain, or hematochezia, with or without fever. However, inflammation may occur in any part of the GI tract including esophagitis and gastritis. Fatalities due to GI perforation have been reported in clinical trials of ipilimumab. Patients should be carefully monitored for GI symptoms that may be indicative of immune-related colitis, diarrhea, or GI perforation. Diarrhea or colitis occurring after initiation of ipilimumab therapy should be evaluated to exclude infectious or alternate etiologies. In clinical trials, immune-related colitis was associated with evidence of mucosal inflammation, with or without ulcerations, and lymphocytic infiltration.

Monitor patients for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, mucus or blood in stool, with or without fever) and bowel perforation (such as peritoneal signs and ileus). In symptomatic patients, rule out infectious etiologies and consider endoscopic evaluation to establish etiology and for persistent or severe symptoms. C.difficile toxin has been detected in several patients with colitis and may be an independent entity or may co-exist with ipilimumab induced inflammatory colitis.

Withhold ipilimumab and/or nivolumab dosing for any patients with enterocolitis pending evaluation; administer anti-diarrheal treatment and, if persistent evaluate with colonoscopy and initiate systemic corticosteroids at a dose of 0.5 mg/kg/day prednisone or equivalent.

Permanently discontinue ipilimumab and nivolumab in patients with severe enterocolitis and initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least one month. In clinical trials, rapid corticosteroid tapering has resulted in recurrence or worsening symptoms of enterocolitis in some patients.

Patients have been treated with anti-TNF agents for persistent colitis not responding to steroids.

Please note autoimmune pancreatitis may cause abdominal pain and should be included in all evaluations. Enteritis may occur occasionally with other autoimmune events including hepatitis, pancreatitis, and endocrine insufficiency, which should be evaluated as clinically indicated.

Immune-mediated Hepatitis and Pancreatitis
Hepatic immune-related AEs were mostly clinically silent and manifested as transaminase or bilirubin laboratory abnormalities. Fatal hepatic failure has been reported in clinical trials of ipilimumab. Serum transaminase and bilirubin must be evaluated before each dose of ipilimumab and nivolumab as early laboratory changes may be indicative of emerging immune-related hepatitis/ pancreatitis and elevations in liver function tests (LFTs) may develop in the absence of clinical symptoms. Increase in LFT or total bilirubin should be evaluated to exclude other causes of hepatic injury, including infections, disease progression, or other medications, and monitored until resolution. Liver biopsies from patients who had immune-related hepatotoxicity showed evidence of acute inflammation (neutrophils, lymphocytes, and macrophages).

Monitor liver function tests (hepatic transaminase and bilirubin levels) and assess patients for signs and symptoms of hepatotoxicity/ pancreatitis before each dose of ipilimumab and nivolumab. In patients with hepatotoxicity, rule out infectious or malignant causes and increase frequency of liver function test monitoring until resolution. Withhold ipilimumab and nivolumab in patients with Grade 2 hepatotoxicity.

Permanently discontinue ipilimumab and nivolumab in patients with Grade 3–5 hepatotoxicity/pancreatitis and administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When liver function tests show sustained improvement or return to baseline, initiate corticosteroid tapering and continue to taper over 1 month. Across the clinical development program for ipilimumab, mycophenolate treatment has been administered in patients who have persistent severe hepatitis despite high-dose corticosteroids.

Immune-mediated Dermatitis
Skin immune-related AEs presented mostly frequently as a rash and/or pruritus. Some patients reported vitiligo associated with ipilimumab administration. Fatal toxic epidermal necrolysis has been reported in clinical trials of ipilimumab.

Monitor patients for signs and symptoms of dermatitis such as rash and pruritus. Unless an alternate etiology has been identified, signs or symptoms of dermatitis should be considered immune-mediated.

Permanently discontinue ipilimumab and nivolumab in patients with Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations. Administer systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent. When dermatitis is controlled, corticosteroid tapering should occur over a period of at least 1 month. Withhold ipilimumab and nivolumab dosing in patients with moderate to severe signs and symptoms.

For mild to moderate dermatitis, such as Grade 2 localized rash and pruritus, treat symptomatically. For persistent Grade 2, Grade 3, or greater, topical steroids may be administered. Administer topical or systemic corticosteroids as indicated if there is no improvement of symptoms within 1 week.

Immune-related Neurological Events
Fatal Guillain-Barré syndrome has been reported in clinical trials of ipilimumab. Patients may present with muscle weakness and myasthenia gravis, cranial nerve palsy (n VII Bell's palsy), and aseptic meningitis, encephalopathy. Unexplained motor neuropathy, muscle weakness, or sensory neuropathy lasting more than 4 calendar days should be evaluated and non-inflammatory causes such as disease progression, infections, metabolic syndromes, nerve entrapment, and medications should be excluded as causes.

Withhold ipilimumab and nivolumab dosing in patients with any evidence of neuropathy pending evaluation.

Monitor for symptoms of motor or sensory neuropathy such as unilateral or bilateral weakness, sensory alterations, or paresthesia. Permanently discontinue ipilimumab and nivolumab in patients with severe neuropathy (interfering with daily activities) such as Guillain-Barré-like syndromes. Institute medical intervention as appropriate for management of neuropathy and other neurologic events. Consider initiation of systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe neuropathies.

Immune-mediated Endocrinopathies
Ipilimumab and/or nivolumab can cause inflammation of endocrine organs including thyroid (Hashimoto's thyroiditis with positive antibodies) and adrenal glands, hypophysitis, hypopituitarism, and resulting thyroid and adrenal insufficiency, low ADH, prolactin, FSH, LH. Hyperthyroid with Graves' disease and positive antibody has been reported. Patients may present with subtle and nonspecific symptoms. The most common clinical presentation includes headache and fatigue. Symptoms may also include visual field defects, behavioral changes, and electrolyte disturbances including hyponatremia and hypotension. Adrenal crisis as a cause of the patient's symptoms should be excluded. Based on the available data with known outcome, most of the patients symptomatically improved with hormone replacement therapy. Long term hormone replacement therapy with HC and Synthroid will typically be required for patients developing hypophysitis/hypopituitarism after treatment with ipilimumab alone or with nivolumab. Some patients have regained partial function following steroid treatment.

Monitor patients for clinical signs and symptoms of hypophysitis, adrenal insufficiency (including adrenal crisis), and hyper- or hypothyroidism. Headache is often the first symptoms of hypophysitis. Patients may present with fatigue, headache, mental status changes, loss of libido, abdominal pain, unusual bowel habits, and hypotension, or nonspecific symptoms which may resemble other causes such as brain metastasis or underlying disease. Unless an alternate etiology has been identified, signs or symptoms of endocrinopathies should be considered immune-mediated and drugs withheld pending evaluation. Patients may demonstrate both central (hypophysitis) and peripheral adrenal and thyroid insufficiency. Evaluation of hypophysitis should include pituitary MRI.

Endocrine evaluation, including TSH, should be performed at baseline prior to initial treatment. Monitor thyroid function tests and clinical chemistries at the start of treatment and hold blood for possible evaluation should clinical events require determining baseline function and anti-thyroid antibodies. In a limited number of patients, hypophysitis was diagnosed by imaging studies

through enlargement of the pituitary gland.

Withhold ipilimumab and nivolumab dosing in patients symptomatic for hypophysitis. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day of prednisone or equivalent and initiate appropriate hormone replacement therapy.

Other Immune-mediated Adverse Reactions, Including Ocular Manifestations
Ocular inflammation, manifested as Grade 2 or Grade 3 episcleritis or uveitis, was associated with concomitant diarrhea in a few patients (< 1%) and occasionally occurred in the absence of clinically apparent GI symptoms. Other presumed immune-related AEs reported include, but were not limited to, arthritis/arthralgias, pneumonitis, pancreatitis, autoimmune (aseptic) meningitis, autoimmune nephritis, pure red cell aplasia, noninfective myocarditis, polymyositis, and myasthenia gravis, of which were individually reported for < 1% of patients.

The following clinically significant immune-mediated adverse reactions were seen in less than 1% of ipilimumab-treated patients in Study 1: nephritis, pneumonitis, pulmonary granuloma resembling sarcoidosis, meningitis, pericarditis, uveitis, iritis, ITP, neutropenia and hemolytic anemia.

Across the clinical development program for ipilimumab, the following likely immune-mediated adverse reactions were also reported with less than 1% incidence: myocarditis, angiopathy, temporal arteritis, vasculitis, polymyalgia rheumatica, conjunctivitis, blepharitis, episcleritis, scleritis, leukocytoclastic vasculitis, erythema multiforme, psoriasis, pancreatitis, arthritis, and autoimmune thyroiditis.

Permanently discontinue ipilimumab and nivolumab for clinically significant or severe immune-mediated adverse reactions. Initiate systemic corticosteroids at a dose of 1 to 2 mg/kg/day prednisone or equivalent for severe immune-mediated adverse reactions.

Administer corticosteroid eye drops to patients who develop uveitis, iritis, or episcleritis. Permanently discontinue ipilimumab for immune-mediated ocular disease that is unresponsive to local immunosuppressive therapy.

Overall, immune-related AEs commonly started within 3 to 10 weeks from first dose, were successfully managed in most instances by omitting doses, discontinuing dosing, and/or through administering symptomatic or immunosuppressive therapy, including corticosteroids, as mentioned above. Immune-related AEs generally resolved within days to weeks in most patients.

6.7 Overlapping toxicities (“shared AEs”)

Although the safety profiles of troriluzole and nivolumab/ipilimumab are different, there are several overlapping side effects. The treating investigator must use judgement to attribute the AEs. The treating investigator may elect to hold all study therapy if they believe the AE is at least possibly related to all of the study drugs, particularly for high grade events.

Shared AEs: The following AEs and laboratory abnormalities are examples of "shared AEs" that are associated with all 3 study agents and, in most cases, should be attributed to all three drugs (possible, probably, or definitely related):

- asthenia
- nausea
- dizziness
- decreased lung function
- diarrhea
- abdominal pain
- pneumonia
- vomiting
- vertigo
- circumoral paresthesia (numbness around the mouth)
- anorexia
- somnolence
- Elevated LFTs
- Neutropenia (rare)

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

Adverse Events Lists

Adverse Event List(s) for troriluzole

The clinical experience of riluzole is relevant given the intention of dosing troriluzole to yield riluzole exposures within the range already well described for riluzole. Riluzole was approved for treatment of ALS in 1995 and US Prescribing Information reflects data from placebo-controlled trials and long-term follow-up. Riluzole is not associated with immune-related adverse events.

Troriluzole is anticipated to have lesser effects on LFTs, as lower molar doses of troriluzole will be expected to yield the riluzole exposures achieved with oral RILUTEK – by virtue of bypassing first-pass metabolism. In addition, peak liver concentrations are expected to be reduced via use of troriluzole as compared to riluzole, which may reduce propensity for LFT changes.

<u>Safety data for riluzole (RILUTEK)</u>
Riluzole doses of up to 200 mg per day were considered well-tolerated and studied in

randomized controlled trials. Among all chronic randomized controlled trials the discontinuation rate due to AEs was approximately 14%.

No AEs occurred greater than 5% in the riluzole group and twice that of placebo. The AEs occurring greater than 5% and at least 2% more than placebo included asthenia (18% vs. 12% placebo) and nausea (14% vs. 9%).

Most common AEs

The most commonly observed AEs associated with the use of RILUTEK more frequently than placebo treated patients were: asthenia, nausea, dizziness, decreased lung function, diarrhea, abdominal pain, pneumonia, vomiting, vertigo, circumoral paresthesia, anorexia, and somnolence. Asthenia, nausea, dizziness, diarrhea, anorexia, vertigo, somnolence, and circumoral paresthesia were dose related.

Approximately 14% (n = 141) of the 982 individuals with ALS who received RILUTEK in premarketing clinical trials discontinued treatment because of an adverse experience. Of those patients who discontinued due to adverse events, the most commonly reported were: nausea, abdominal pain, constipation, and ALT elevations. In a dose response study in ALS patients, the rates of discontinuation of RILUTEK for asthenia, nausea, abdominal pain, and ALT elevation were dose related.

Incidence in Controlled ALS Clinical Studies

Table 1 lists treatment-emergent signs and symptoms that occurred in at least 2% of patients with ALS treated with RILUTEK (n=794) participating in placebo-controlled trials and were numerically greater in the patients treated with RILUTEK 100 mg/day than with placebo or for which a dose response relationship is suggested.

Table 1
Adverse Events Occurring in Placebo-Controlled Clinical Trials
†Percentage of patients reporting events

Body System / Adverse Event†	Riluzole 50 mg/day (N=237)	Riluzole 100 mg/day (N=313)	Riluzole 200 mg/day (N=244)	Placebo (N=320)
Body as a Whole				
Asthenia	14.8	19.2	20.1	12.2
Headache	8.0	7.3	7.0	6.6
Abdominal pain	6.8	5.1	7.8	3.8
Back pain	1.7	3.2	4.1	2.5
Aggravation reaction	0.4	1.3	2.0	0.9
Malaise	0.4	0.6	1.2	0.0
Digestive				
Nausea	12.2	16.3	20.5	10.6
Vomiting	4.2	4.2	4.5	1.6
Dyspepsia	2.5	3.8	6.1	5.0
Anorexia	3.8	3.2	8.6	3.8
Diarrhea	5.5	2.9	9.0	3.1
Flatulence	2.5	2.6	2.0	1.9
Stomatitis	0.8	1.0	1.2	0.0
Tooth disorder	0.0	1.0	1.2	0.3
Oral Moniliasis	0.4	0.6	1.2	0.3
Nervous				
Hypertonia	5.9	6.1	5.3	5.9
Depression	4.2	4.5	6.1	5.0
Dizziness	5.1	3.8	12.7	2.5
Dry mouth	3.0	3.5	2.0	3.4
Insomnia	2.1	3.5	2.9	3.4
Somnolence	0.8	1.9	4.1	1.3
Vertigo	2.5	1.9	4.5	0.9
Circumoral paresthesia	1.3	1.6	3.3	0.0
Skin and Appendages				
Pruritus	3.8	3.8	2.5	3.1
Eczema	0.8	1.6	1.6	0.6
Alopecia	0.0	1.0	1.2	0.6
Exfoliative dermatitis	0.0	0.6	1.2	0.0
Respiratory				
Decreased lung function	13.1	10.2	16.0	9.4
Rhinitis	8.9	6.4	7.8	6.3
Increased cough	2.1	2.6	3.7	1.6
Sinusitis	0.4	1.0	1.6	0.9
Cardiovascular				

Hypertension	6.8	5.1	3.3	4.1
Tachycardia	1.3	2.6	2.0	1.3
Phlebitis	0.4	1.0	0.8	0.3
Palpitation	0.4	0.6	1.2	0.9
Postural hypotension	0.8	0.0	1.6	0.6
Metabolic and Nutritional Disorders				
Weight loss	4.6	4.8	3.7	4.7
Peripheral edema	4.2	2.9	3.3	2.2
Musculoskeletal System				
Arthralgia	5.1	3.5	1.6	3.4
Urogenital System				
Urinary tract infection	2.5	2.6	4.5	2.2
Dysuria	0.0	1.0	1.2	0.3

Less Common AEs

- Neutropenia. Rare cases of neutropenia (3 out of 4000 subjects in USPI) have been reported
- Interstitial lung disease, including hypersensitivity pneumonitis, has occurred.
- Liver Injury. Lab abnormalities associated with riluzole consist of elevated transaminases that typically are below 5x upper limit of normal (ULN) and often resolve while on treatment. Experience with incidents of alanine aminotransferase (ALT) increasing >5xULN are limited, insofar as the USPI recommends immediate drug discontinuation. Effects on transaminases show a dose response. Importantly, total daily doses of 50 mg were not associated with increased rates of marked ALT elevations (>5xULN) compared to placebo (1.3% vs. 2.1%, respectively) and such occurrences had a later onset than placebo (median 254 vs. 219 days). Total daily doses of 100 mg and 200 mg had greater rates of marked ALT elevations than placebo, and the median occurrence was within 60 days.

Higher dose studies in ALS

Phase 1 studies of riluzole safety explored much higher doses. Twelve subjects each were administered single oral doses of 150mg, 200 mg, 250 mg and/or 300 mg. At doses of 250 mg and 300 mg, riluzole administration was associated with dose-dependent dizziness/vertigo and buccofacial or manual paresthesia.

Troriluzole Phase Ib study with nivolumab

Fourteen patients with advanced solid tumors (melanoma=3, NSCLC=3, renal cell cancer=2, NSCLC=2, head and neck cancer=2) were treated with escalating doses of troriluzole.[30] Patients were exposed to troriluzole doses from 140mg to 560mg total daily dose. The most common TEAEs (all grades) occurring in >40% of patients were transaminitis, increased lipase and nausea. DLT occurred in 3 patients: 1) grade 3 anorexia, 2) grade 3 fatigue and, 3) atrial fibrillation. The MTD was determined to be troriluzole 140 mg QAM + 280 mg PO QPM.

Adverse Event List for Ipilimumab and Nivolumab

Ipilimumab and Nivolumab are known to have adverse events and immune-related adverse events, commonly involving the skin, liver, gut, and less commonly the pancreas, nervous system, heart, lungs, and kidneys. Please refer to the Investigators Brochures and package inserts for the comprehensive list of adverse events.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Adverse Event Reporting

In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the PI.

Investigators **must** report to the PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

Adverse Event Reporting Guidelines

All participating sites will report AEs to the Sponsor-Investigator per DF/HCC requirements, and the IRB of record for each site as applicable per IRB policies. The table below indicates which events must be reported to the DF/HCC Sponsor-Investigator.

Attribution	DF/HCC Reportable Adverse Events (AEs)				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required∞	5 calendar days	5 calendar days#	5 calendar days	24 hours*

If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.

* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, events must be reported within 1 business day of learning of the event.

∞Additional Protocol-Specific Reporting for Select Neurotoxicities

For this protocol only, select grade 2 neurologic toxicity lasting 4 days or more, with or without medical intervention (listed below) that are at least possibly related to study require expedited reporting within 5 calendar days

- Ataxia
- Central nervous system necrosis (use this term for radiation necrosis)
- Cognitive disturbance
- Concentration impairment
- Depressed level of consciousness
- Dizziness
- Encephalopathy
- Guillain-Barre syndrome
- Headache
- Intracranial hemorrhage
- Ischemia cerebrovascular
- Leukoencephalopathy
- Memory impairment
- Presyncope
- Reversible posterior leukoencephalopathy syndrome (PRES)
- Stroke
- Somnolence
- Confusion (categorized under Psychiatric disorders)

Note: the select neurotoxicities above also meet the definition of a DLT in the Phase Ib portion.

Protocol-Specific Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the DF/HCC Sponsor-Investigator. However, they still must be recorded through the routine reporting mechanism (i.e. case report forms).

CTCAE System Organ Class	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments

Investigations	Laboratory abnormality	1-4	See comment	any	Laboratory abnormality without any clinical symptomatology (e.g. asymptomatic elevation of lipase). If the event results in hospitalization or prolongs hospitalization, the exclusion does not apply.

7.4 Reporting to the Food and Drug Administration (FDA)

The Sponsor-Investigator will be responsible for all communications with the FDA. The Sponsor-Investigator will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.5 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.6 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

Treatment-emergent AEs should continue to be monitored during the follow-up period (until resolution). New AEs during follow up that are not related to treatment should not be recorded

8. PHARMACEUTICAL INFORMATION

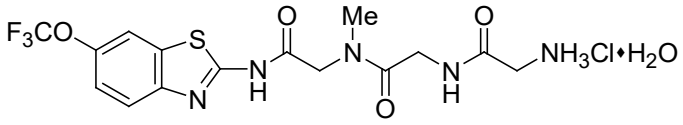
A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

8.1 Troriluzole

Description

Troriluzole is also known as FC-4157, BHV-4157, and trigriluzole. Refer to the Investigational Drug Brochure (IDB).

The active ingredient of troriluzole is a member of the benzothiazole chemical class. The chemical name is 2-Amino-N-((methyl-[(6-trifluoromethoxy-benzothiazol-2-ylcarbamoyl)-methyl]-carbamoyl)-methyl)-acetamide monohydrate monohydrochloride. The molecular formula is $C_{15}H_{16}F_3N_5O_4S.HCl.H_2O$. The chemical structure is seen in the figure below:



Troriluzole, **2**

Drug Interactions: Clinical drug interaction studies for troriluzole have not been conducted yet. Troriluzole, itself, is not expected to interfere with drug metabolism and its cleavage via proteases render it unlikely to be affected significantly by liver cytochrome P450 inhibitors. It is not an inhibitor or CYP3A4, CYP1A2, or CYP2D6.

Half-life in blood is <0.5 hours (in vitro).

Form

The formulation for clinical development is a common blend capsule of 140 mg of troriluzole (dose based on study design) administered orally. The capsule is a formulated common blend of troriluzole with standard excipients from a dry granulation, roller compaction process is filled in white opaque hard gelatin Size 1 capsules containing 140 mg of troriluzole. This formulation is intended for both clinical studies and commercial use. The excipients are mannitol, microcrystalline cellulose, hydroxypropyl cellulose, crospovidone, colloidal silicon dioxide, and magnesium stearate. The capsules are supplied in 35-count, 75 cc, wide mouth round white high density polyethylene bottles with a polyester coil and induction sealed 38 mm white child-proof screw cap closures for clinical use.

Visually matching placebos containing microcrystalline cellulose are used throughout the clinical trials.

Storage and Stability

Investigational drug will be stored in the Research Pharmacy at each site. The investigational pharmacists will be responsible for preparation, dispensing, disposal, and drug accountability.

Formulated blend capsules are stored at room temperature between 20°C and 25°C (68°F to 77°F) with temporary excursions permitted between 15°C and 30°C (59°F to 86°F).

Stability of formulated blend capsules and placebo capsules at 18 months also show no significant change. Stability studies are ongoing and are expected to extend the retest period of clinical supplies.

Compatibility

N/A

Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

Availability

The investigational drug and placebo will be supplied by Biohaven Pharmaceuticals. The drug substance is manufactured and purchased commercially from Anthem Biosciences.

Preparation

N/A

Administration

Troriluzole or placebo will be self-administered by the patient orally with continuous BID dosing. Ideally, BID dosing will be as close to Q 12 hours (± 2 hours) apart as possible (minimum 10 hours apart). Troriluzole may be taken either with food or on an empty stomach.

Ordering

Each site will order drug from Biohaven Pharmaceuticals using the form provided. Drug should be ordered at least 7 days in advance of anticipated dispensing.

Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

Destruction

Expired, empty and partially empty containers of study drug will be disposed of/destroyed in accordance with institutional policies and procedures. Unused containers of study drug will not be returned to the study sponsor.

8.2 Ipilimumab

Ipilimumab (YERVOY), commercial supply.
Refer to the package insert for agent information.
http://packageinserts.bms.com/pi/pi_yervoy.pdf

8.3 Nivolumab

Nivolumab (OPDIVO), commercial supply.
Refer to the package insert for agent information.
http://packageinserts.bms.com/pi/pi_opdivo.pdf

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

The following samples will be collected as per the Study Calendar (mandatory unless specified as optional):

a) Archival tissue at baseline (mandatory)
b) Serial blood samples for PBMCs and serum on Days 1, 43, 85, 169, and 253 (mandatory)
c) Fresh biopsy of extracranial disease (optional, Day 0 and 43)
d) Intracranial tissue and CSF (optional, at time of clinically indicated brain surgery in a subset of patients)

All biomarker studies are ancillary/exploratory. We will compare samples from responders and non-responders to test for differences in correlatives. When available, we will compare pre/post paired treatment biopsies of extracranial tumors and brain/body paired biopsies from the same patient. When CSF is available, we will compare paired CSF/serum from the same patient.

The following studies will be performed:

IHC on archival tumor samples. We hypothesize that nearly 100% of melanomas will have GRM1 expression, providing rationale for exploring glutamate modulation as a target in melanoma. IHC will also be used to determine PD-L1 expression, the degree of TILs, and other immune effector cells, and on-treatment biopsies will be examined for change in TILS and decrease in signal transduction in key pathways (e.g. MAPK, Pi3K/AKT).

Flow cytometry on PBMCs. Immunophenotypic analysis of immune cells and markers will be assessed by multiparameter flow cytometry. We hypothesize that responders will display more favorable T cell population ratios, markers of activation, and differentiation, and fewer MDSCs.

Multiplex cytokine analysis on serum and CSF. We hypothesize that responders will demonstrate a decrease in the expression of VEGF, IL-8, CD34, CCL2, and M-CSF.

Gene expression analysis by Nanostring. We hypothesize that responders will have a higher immune-related gene expression profile.

Tumor genomic profiling. We will determine if activating mutations in B-RAF or N-RAS, or PTEN inactivation (or activating B-RAF mutations and PTEN inactivation together) in the pre-treatment tumor samples correlates with response to therapy.

Exosome analysis. We will examine exosome contents and hypothesize that post-treatment serum samples will contain fewer suppressive cytokines than pre-treatment samples.

9.2 Laboratory Correlative Studies

Please refer to the *Sample Collection Laboratory Manual* for handling and shipping instructions. Samples will be collected and shipped to:

Andrew Zloza
1735 West Harrison Street
Cohn Research Building - Room 447
Rush University
Chicago, Illinois 60612
Phone: (312) 942-2321
Fax: (312) 942-3602
Email: andrew_zloza@rush.edu

The samples will be partitioned for analysis in two labs. Some samples will remain in the Zloza lab for analysis. A portion of the samples will be performed shipped to the Chen lab for analysis at Rutgers University.

10. STUDY CALENDAR

10.1 Scheduling windows

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy. Scans and x-rays must be done <4 weeks prior to the start of therapy. If the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within + 7 days of the protocol-specified date for Days 1-309, and within + 14 days of the protocol-specified date for Days 337-673.

	Baseline	Cycles 1-4: Induction				Cycles 5-13			End of Treatment (EOT) Cycle 14	Follow-Up Visit #1	Follow-Up Visits #2-4[i]	Final Follow-Up Visit[j]	EDC Timepoints
		Day 1	Day 22	Day 43	Day 64	Day: 85 169 253	Day: 113 197 281	Day: 141 225 309	Day 337 or sooner	Within 30 days days post EOT	Day: 421 505 589	Day 673 or sooner	
Troriluzole or placebo[T] (continuous oral twice daily dosing)		T----------T											Medication Diary Documentation Day 43, 85, 169, 253, EOT
Ipilimumab and Nivolumab		I+N	I+N	I+N	I+N								Day 1, 22, 43, 64
Nivolumab						N	N	N	N				Day 85, 113, 141, 169, 197, 225, 253, 281, 309
Informed consent	X												N/A
Demographics	X												Baseline
Medical history, including BRAF mutational status	X												Baseline
Physical exam	X	X	X	X	X	X	X	X	X	X	X	X	Baseline
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	Baseline
Height	X												Baseline

	Baseline	**Cycles 1-4: Induction**				**Cycles 5-13**			**End of Treatment (EOT) Cycle 14**	**Follow-Up Visit #1**	**Follow-Up Visits #2-4[i]**	**Final Follow-Up Visit[j]**	**EDC Timepoints**
		Day 1	Day 22	Day 43	Day 64	Day: 85 169 253	Day: 113 197 281	Day: 141 225 309	Day 337 or sooner	Within 30 days days post EOT	Day: 421 505 589	Day 673 or sooner	
Weight	X	X	X	X	X								Baseline
Performance status	X	X				X			X	X			Baseline, EOT
CBC w/diff, plts and CMP[a]	X	X	X	X	X	X	X	X	X	X	X	X	N/A
Gamma-glutamyl transferase (GGT), LDH, amylase, lipase, TSH, free T4[b]	X					X			X	X			GGT only: Baseline, Day 85, 169, 253, 337
B-HCG[c], pro-BNP, troponin-T	X												N/A
EKG	X					X							Baseline, Day 85, 169, 253. If EKG is performed during any other Cycle, it must be captured in EDC.
MRI Brain (with and without gadolinium contrast) for intra-	X			X		X			X				Baseline, Day 43, 85, 169, 253. If Intracranial Tumor Measurements are performed

	Baseline	**Cycles 1-4: Induction**				**Cycles 5-13**			**End of Treatment (EOT) Cycle 14**	**Follow-Up Visit #1**	**Follow-Up Visits #2-4[i]**	**Final Follow-Up Visit[j]**	**EDC Timepoints**
		Day 1	Day 22	Day 43	Day 64	Day: 85 169 253	Day: 113 197 281	Day: 141 225 309	Day 337 or sooner	Within 30 days days post EOT	Day: 421 505 589	Day 673 or sooner	
cranial tumor measurements [d]													during any other Cycle, it must be captured in EDC.
CT CAP (*or PET/CT*) for extra-cranial tumor measurements [e]	X					X			X				Baseline, Day 85, 169, 253. If Extracranial Tumor Measurements are performed during any other Cycle, it must be captured in EDC.
Research blood samples[f]	X			X		X			X				N/A
Archival tumor specimen (6-10 unstained slides)	X												N/A
Research fresh tumor biopsy and additional blood draw (optional) [g]	X			X									N/A
Concurrent Medications		X----------X											Final Follow-Up Visit (Steroid use only)
Adverse Event Evaluation		X----------X											All visits.

	Baseline	Cycles 1-4: Induction				Cycles 5-13			End of Treatment (EOT) Cycle 14	Follow-Up Visit #1	Follow-Up Visits #2-4[i]	Final Follow-Up Visit[j]	EDC Timepoints
		Day 1	Day 22	Day 43	Day 64	Day: 85 169 253	Day: 113 197 281	Day: 141 225 309	Day 337 or sooner	Within 30 days days post EOT	Day: 421 505 589	Day 673 or sooner	
Other correlative studies on tumor tissue – if brain surgery occurs during the study period[h]		X----------										----------X	If brain surgery is performed, EDC should capture date, and which tumor was removed.
Concurrent radiation therapy		X----------										----------X	If brain or body RT is performed, EDC should capture start/end dates, # fractions, and total dose (cGy) to each tumor that was irradiated.
Survival		X----------										----------X	N/A

Study Drug Dose and administration schedule:

T: Troriluzole [FC-4157, BHV-4157] or placebo. Safety Run-In cohort will start at 140mg PO Q AM and 280mg PO QPM only open label Troriluzole. This dose level may change based on participant cohort and MTD determined in Safety Run-In (Section 5).

I+N: Ipilimumab + nivolumab induction: ipilimumab 3 mg/kg IV Q3 weeks and nivolumab 1 mg/kg IV Q3 weeks.

N: Nivolumab: nivolumab 480mg IV Q4 weeks.

Footnotes:

a: Comprehensive metabolic panel (CMP): Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

b: TSH with reflex to free T4 is acceptable replacement for TSH and free T4.

c: Serum pregnancy test (women of childbearing potential).

d: MRI Brain (with and without gadolinium contrast) for intra-cranial tumor measurements with thin cuts. Slice thickness must be 2 mm or less—specify when ordering.

e: PET/CT scan is an allowable alternative to CT scan if approved by Sponsor-Investigator. Tumor assessments will be performed as per standard of care during Days 421-589 and will be captured on CRFs.

f: Research blood samples. At each time point indicated, 2 green top (heparin) tubes (10 ml each) will be collected for biomarker/correlative studies listed in Section 9.

	Baseline	Cycles 1-4: Induction				Cycles 5-13			End of Treatment (EOT) Cycle 14	Follow-Up Visit #1	Follow-Up Visits #2-4[i]	Final Follow-Up Visit[j]	EDC Timepoints
		Day 1	Day 22	Day 43	Day 64	Day: 85 169 253	Day: 113 197 281	Day: 141 225 309	Day 337 or sooner	Within 30 days days post EOT	Day: 421 505 589	Day 673 or sooner	

g: Fresh biopsy of extracranial disease (optional). The second biopsy may occur at any time between Days 43 and 64 to facilitate scheduling. Patients will also have 2 CPT top tubes (10 mL tubes) drawn on the day of the fresh tumor biopsies. Biopsy and shipping must occur Monday – Thursday.

h: If patient is having a craniotomy per standard of care, tumor sample from brain metastasis and 1-2 green top vials of CSF will be collected when feasible.

i: Follow-up visits #2-4. If patients discontinue treatment early, they should be evaluated in-person every 84 days (or more frequently as indicated) until 5 Follow-Up visits have been completed.

j: Final In-Person visit. Survival data will continue to be collected for 3 years, unless patient dies or withdraws consent to be followed. The study team will contact participants by phone every 12 weeks (+/- 14 days) to assess survival.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Intracranial tumors and Extracranial tumors

Assessment of extracranial disease (by CT scan or other approved modalities) and intracranial disease (by MRI scan with a minimum of 3 mm cuts) will be performed per the schedule in Section 10, Study Calendar.
Investigators may obtain more frequent scans as medically indicated.

Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[39] for all extracranial lesions and modified RECIST 1.1 for all brain lesions (see Section 11.3).

Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Definitions

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

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle (3 weeks) of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

Disease Parameters

Measurable disease, **intracranial (brain lesions).** Measurable brain lesions are defined in this study as those that can be accurately **measured in at least one dimension (longest diameter to be recorded) as ≥5 mm with MRI**. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Brain metastases that have previously been treated with radiation therapy are not considered measurable, unless they have grown and the treating investigator considers them to be refractory to radiation.

Measurable disease, **extracranial** lesions (including skull, neck, chest abdomen, pelvis, and extremities). Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor

measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that that have previously been treated with radiation therapy are not considered measurable, unless they have grown and the treating investigator considers them to be refractory to radiation.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <5 mm in the brain or <10 mm in the body, or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable).

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ (modification to allow up to 5 lesions in brain) and 8 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.2 Methods for Evaluation of Disease

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

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

CT scans. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness.

MRI scans with "thin cuts." MRI is required for assessment of brain metastases with **slices of 2mm or less. Because this study allows a minimum lesion size of 5mm in the brain, all brain MRIs must be performed with "thin cuts" (slice thickness required to be no greater than 2 mm).** MRI also appropriate in certain additional situations (*e.g.* for extremities). The technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease

on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

MIBG (meta-iodobenzylguanidine). The following is recommended, to assure high quality images are obtained.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Participants and/or parents are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150 μCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A

large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.3 Response Criteria: modified RECIST version 1.1

Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1);[39] however there will be **two modifications for assessment of brain lesions on this protocol**:

1) modification to allow up to five target lesions in the brain (all other organs continue to have a maximum of 2 target lesions), and the maximum number of target lesions globally may be up to 10 lesions.

2) to include target lesions in the brain measuring ≥5 mm in their longest diameter as seen on thin cut MRI (slice thickness ≤2 mm), as described previously in other brain

metastases trials.[3,40,41]

Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (<10 mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s).

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or

flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Evaluation of BOR

Target Lesions	Non-Target Lesions	New Lesions*	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-CR/Non-PD/not evaluated	No	PR
SD	Non-CR/Non-PD/not evaluated	No	SD
PD	Any	Yes or No	PD
Any	PD**	Yes or No	PD
Any	Any	Yes	PD

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

11.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first

date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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

11.5 Progression-Free Survival

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression in the intracranial or extracranial sites or death due to any cause. Herein, the term "PFS" refers to PFS in both the intracranial and extracranial compartments (global). The follow-up of participants alive without disease progression is censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.6 Response Review

Central review is not required.

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

Method

The DF/HCC Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

Responsibility for Data Submission

Investigative sites are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

12.2 Data Safety Monitoring

12.2.1 The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from the randomized portion of this study. The Board is chaired by a medical oncologist from outside of DF/HCC and its membership composed of internal and external institutional representation. Information that raises any questions about participant safety or protocol performance will be addressed by the Sponsor-Investigator, statistician and study team. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the study.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual; treatment regimen information; all adverse events and serious adverse events reported across all sites by category; summary of any deaths on study; audit results; and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.2.2 The DF/HCC Data Safety Monitoring Committee (DSMC) will review and monitor study progress, toxicity, safety and other data from the safety run-in phase of this study. The DSMC meets monthly to review protocols and is chaired by a medical oncologist appointed by the Senior VP for Research. The DSMC will typically review a protocol every 3, 6, or 9 months based on protocol specifics. Information that raises any questions about participant safety or protocol performance will be addressed by the Sponsor-Investigator, statistician and study team. Should any major safety concerns or issues with data compliance arise, the DSMC will offer recommendations regarding whether or not to suspend the study.

12.3 Multi-Center Guidelines

This protocol will adhere to DF/HCC Policy MULTI-100 and the requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Sponsor-Investigator, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix B.

12.4 Collaborative Agreements Language

N/A

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

It was recently shown that patients with small asymptomatic brain metastases can be treated with medical therapy rather than upfront radiation therapy.[3] The response rate in this study was 48% and the intracranial and the extracranial response rates were largely concordant. Progression-free survival at 6 months (PFS6) was 61%. However, many patients progressed within the first 6 months. Therefore, freedom from progression at the 6-month mark is a clinically important and

measurable outcome.

We propose a multi-center, double-blind, randomized, phase II signal-detection trial with a non-randomized safety run-in to assess the efficacy and safety of adding troriluzole to ipilimumab/nivolumab induction and nivolumab maintenance in patients with melanoma that has metastasized to the brain. Patients will be randomly assigned in equal proportions to ipilimumab/nivolumab/placebo induction followed by nivolumab/placebo maintenance (INP) or ipilimumab/nivolumab/troriluzole induction followed by nivolumab/troriluzole maintenance (INT). The randomization will be stratified by prior BRAF-directed therapy (yes/no) and prior therapy with anti-PD-1 treatments (yes/no).

Safety Run-in: Up to three cohorts of patients will be treated in a 3+3 design with the triple drug combination to provide dosing information and an early assessment of safety. A starting dose of troriluzole at 140 mg/280 mg will be used; two fallback doses will also be investigated if the starting dose proves too toxic.

Cohort de-escalation table during safety run-in:

Dose Level	**Troriluzole Dose**
Starting cohort	140 mg PO QAM and 280 mg PO QPM
Cohort -1	140 mg PO BID
Cohort -2	140 mg PO daily
If ≥2 DLTs on Cohort -2	Permanently stop the trial

At the end of each cohort of the safety run-in, enrollment will pause for a comprehensive review of the safety data including dose-limiting toxicity (DLT). If fewer than two patients within a cohort have DLTs then that dose level will be considered the MTD and enrollment to the randomized trial will begin. If two or more patients within a safety cohort have DLTs, then the dose will either be de-escalated, or the trial will stop. A participant will be considered to have completed the DLT period with no dose limiting toxicities only if they have taken ≥ 80% of their assigned Troriluzole doses in the DLT period, which is the first 6 weeks of treatment.

Following the Safety Run-in, all participants will be enrolled on the randomized portion of the study.

Primary Endpoint. The primary endpoint of the study is global progression-free survival in patients with melanoma brain metastases who are treated with INT vs. INP. Of particular interest will be PFS at 6 months.

PFS is defined as the time from random assignment to the earlier of death or documented disease progression in the intracranial or extracranial compartments. The follow-up of patients who have neither died nor progressed will be censored at the date of the last follow-up visit.

Secondary Endpoints. The secondary objectives and their associated endpoints evaluate other important clinical outcomes, including overall survival (OS), intracranial response rate (RR) and

PFS, extracranial RR and PFS, and safety (number of participants experiencing adverse events, particularly neurologic events). We will also investigate:

- tolerability (number of induction cycles administered, number of maintenance cycles administered)
- use of corticosteroids for management of symptomatic cerebral edema (number of participants)
- use of corticosteroids for management of immune related adverse events (number of participants who require prednisone ≥1 mg/kg or equivalent)
- frequency of clinically-indicated stereotactic radiation therapy to the brain on study (number of patients, number of instances and number of lesions irradiated)
- frequency of clinically-indicated surgical intervention to the brain on study (number of participants)

Planned subgroup analyses according to baseline characteristics:

- Prior anti-PD-1 exposure (yes vs. no – if yes then adjuvant vs. metastatic setting)
- Prior ipilimumab exposure (yes vs. no)
- Age (<65 years vs 65 years or older)
- ECOG (0 vs 1)
- LDH at baseline (normal vs. elevated)
- Number of brain metastases (<3 vs 3 or more)
- Brain-directed stereotactic radiation therapy prior to study entry (yes vs. no)
- Craniotomy prior to study entry (yes vs. no)

Planned subgroup analyses according to on-study events:

- On-study brain-directed stereotactic radiation therapy (yes vs. no)

<u>Exploratory Endpoints:</u>

- associations between BRAF/NRAS mutation status and response endpoints, as well as tissue biomarker profiles between paired tissues from extracranial and intracranial metastases from individual patients, where available
- associations between peripheral blood immune cell subpopulations (which may include but is not limited to T-cell, NK, B-cell, MDSC, and serum soluble factors with clinical endpoints and/or the occurrence of adverse events

13.2 Sample Size, Accrual Rate and Study Duration

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	1	+	2	=	3
Not Hispanic or Latino	39	+	66	=	105
Ethnic Category: Total of all subjects	40	+	68	=	108
Racial Category					

American Indian or Alaskan Native	0	+	0	=	0
Asian	1	+	2	=	3
Black or African American	0	+	0	=	0
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	39	+	66	=	105
Racial Category: Total of all subjects	40	+	68	=	108

Sample Size. The total sample size for this investigation is between 6 and 108 patients, which includes 6 - 18 patients in the safety run-in, and up to 90 patients in the randomized portion.

Sample size estimates for the randomized trial are based on a proportional hazards mixture cure model with type-I error of 0.1, uniform accrual over 24 months, and 12 additional months of follow-up. The six-month global PFS proportion in the INP arm is assumed to be 0.6^3 and follows an exponential distribution; it is also assumed that approximately 30% of patients in the INP arm and 50% of patients in the INT arm are cured (odds ratio = 2.33). Under these assumptions, there would be 80% power to detect a 6-month PFS rate of approximately 0.8 in the INT arm (hazard ratio = 0.557).[42]

Accrual rate. Assuming accrual of about 3 patients per month across 6 sites and an equal allocation between experimental and control arms, 36 patients will be accrued within one year.

Study duration. Assuming unequal protocol activation times and holding accrual for analysis of safety run-in, study duration will be 4-5 years.

13.3 Stratification Factors

The randomization will be stratified by prior BRAF-directed therapy (yes/no) and prior therapy with anti-PD-1 treatments (yes/no).

13.4 Interim Monitoring Plan

Safety Run-in: The DLT observation period is 6 weeks. After all patients in a dose cohort have been followed for 6 weeks or had dose-limiting toxicity (DLT), enrollment will pause for review of the safety data. The safety reviews will decide whether to declare an MTD and proceed with the randomized trial, adjust the dose, or stop enrollment.

Early stopping rule for futility: An interim look for futility will take place when approximately 45 patients have been enrolled and treated on the Phase II portion of the trial (a total of 15 PFS events have occurred). A proportional hazards mixture cure model (Section 13.5) will be fit to the data at the time of the interim analysis and will be stratified by the randomization factors; randomized treatment will be the only predictor in this model. If the observed hazard ratio (INT vs. INP) from the latency portion of the model is 0.96 or larger then study enrollment will stop and the trial will cease due to futility.

Early stopping rule for neurotoxicity: Sequential boundaries will be used to monitor the rate of neurotoxicity (Section 7.3 Adverse Event Reporting) in the first 45 patients enrolled and treated in the Phase II portion of the trial. Accrual will be halted if excessive numbers of neurotoxicities are seen, that is, if the number of neurotoxicities is equal to or exceeds b_n out of n patients (see table). This is a Pocock-type stopping boundary that yields the probability of crossing the boundary = 0.1 when the rate of neurotoxicity is equal to 0.20.[43] Accrual will be halted if the number of neurotoxicities is equal to or exceeds b_n out of n patients, and the DSMB or DSMC may permanently stop the trial.

Table for neurotoxicity stopping rule:

Number of Patients, *n*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Boundary, b_n	-	-	3	3	4	4	5	5	5	6	6	6	7	7	7	7	8	8	8	9
Number of Patients, *n*	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Boundary, b_n	9	9	9	10	10	10	11	11	11	11	12	12	12	12	13	13	13	13	14	14
Number of Patients, *n*	41	42	43	44	45															
Boundary, b_n	14	14	15	15	15															

13.5 Analysis of Primary Endpoints

Primary Endpoint: The primary endpoint of the study is global progression-free survival in patients with melanoma brain metastases who are treated with troriluzole/ipilimumab/nivolumab (INT) vs. ipilimumab/nivolumab (INP).

Analysis of the primary endpoint will be based on the proportional hazards mixture cure model (PHMC) with treatment (INT/INP) as the only predictor. Modeling of parameter estimates of survival and cure will be semiparametric with bootstrapped standard errors and stratified by the randomization factors. Analyses will be conducted using the *smcure* package from R, the PSPMCM macro from SAS, or any modification to these packages available at the time of analysis. Inference will be based on Z-values and associated p-values. Cure proportions will be based on estimation from the incidence portion of the model and metrics of PFS from patients who are not cured will be based on the latency portion of the model. Comparisons of cure rates will be expressed as odds ratios with confidence intervals, and comparisons of PFS will be expressed as hazard ratios with confidence intervals. The distributions of events for INT and INP will be summarized using marginal survival function curves. Point estimates at 6 months will be presented with confidence intervals.

13.6 Analysis of Secondary Endpoints

Overall Survival
Overall Survival (OS) is defined as the time from randomization (or registration) to death due to

any cause, or censored at date last known alive. Estimates of overall survival will also be from a PHMC model (Section 13.5). Long-term survival estimates and comparisons will be based on the incidence portion of the model; hazards of death will be based on the latency portion of the model. The distribution of deaths will be summarized using marginal survival function curves.

Intracranial or Extracranial Response

The proportion of patients in each treatment arm with intracranial response (RECIST) will be presented with exact, 90% binomial confidence intervals. The randomized treatment arms will be compared using Fisher's exact test. Comparable comparisons will be conducted for extracranial response (RECIST).

Intracranial or Extracranial PFS

Extracranial PFS is defined as the time from first dose of study therapy to documented extracranial progression (per RECIST) or death, whichever occurs first. Intracranial PFS is defined as the time from first dose of study therapy to documented intracranial progression or death, whichever occurs first. For both endpoints, the follow-up of patients who have neither died nor progressed at the time of analysis will be censored at the date of last adequate disease assessment. Treatment comparisons of intracranial or extracranial PFS will be based on PHMC methods outlined in Section 13.5. Point estimates at 6 months will be presented with confidence intervals.

Safety Data

Adverse events will be summarized for all patients having received one or more doses of study therapy and reported according to CTCAE (Version 5.0). The data for each dose cohort in the dose escalation will be summarized separately as will the safety data from the randomized portion of the trial. The proportions of patients in the randomized trial with grade-3 or higher toxicities will be presented with 90% exact binomial confidence intervals. The incidence of events that are new or worsening from the time of first dose of therapy will be summarized according to system organ class and/or preferred term, severity (based on CTCAE grade), type of adverse event, and relation to study treatment. Deaths reportable as SAEs and non-fatal serious adverse events will be listed by patient and tabulated by primary system organ class, and type of adverse event. Of particular interest are serious neurologic events such as seizure, intracranial hemorrhage, and radiation necrosis.

Tolerability

Drug administration will be summarized for all patients having received one or more doses of study therapy. Tolerability will be described by the number of induction cycles administered, the number of maintenance cycles administered, and the frequency of discontinuation of therapy due to toxicity.

Planned Subgroup Analyses

Pre-specified subgroup analyses according to baseline characteristics will be conducted using PHMC methods and will be based on the statistical interaction of treatment assignment and the characteristic. Statistical inference will be based on the interaction p-value. Forest plots will be used to show the results graphically.

Preplanned sub-analyses will be conducted on patients who received on-study brain-directed stereotactic radiation ("radiation salvage subgroup"). Tumor response in the non-irradiated brain lesions and overall survival will be described.

Preplanned sub-analyses will be conducted on patients who required corticosteroids to treat either cerebral edema or immune-related adverse events ("steroid-treated subgroups").

Due to the potential for guarantee-time bias in these analyses, a conditional landmark approach will be used to evaluate these data.

Exploratory Analyses
Biomarkers of blood and tissue: Analyses of blood and tissue biomarkers will be based on descriptive statistics, as appropriate for the biomarker. To examine response according to pre-treatment levels of biomarkers, the study sample will be divided retrospectively according to response and summarized descriptively for the resulting groups. These will be compared using Wilcoxon rank-sum tests for markers measured on a continuous scale, or Fisher's exact tests for those measured on a categorical scale. Where appropriate, visualization of the relationship between baseline marker levels and the distributions of PFS or OS will employ marginal survival function estimates stratified by biomarker levels.

13.7 Reporting and Exclusions

Evaluation of Toxicity
All participants will be evaluable for toxicity from the time of their first treatment.

Evaluation of the Primary Efficacy Endpoint
The analysis of the primary endpoint will be conducted using the intention-to-treat principal, meaning all randomized patients will be evaluated according to the treatment assigned at the time of enrollment.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

15. REFERENCES

1. Goyal S, Silk AW, Tian S, et al. Clinical Management of Multiple Melanoma Brain Metastases: A Systematic Review. *JAMA Oncology.* 2015;1(5):668-676.
2. Fischer GM, Jalali A, Kircher DA, et al. Molecular Profiling Reveals Unique Immune and Metabolic Features of Melanoma Brain Metastases. *Cancer Discovery.* 2019;9(5):628.
3. Tawbi HA, Forsyth PA, Algazi A, et al. Combined Nivolumab and Ipilimumab in Melanoma Metastatic to the Brain. *New England Journal of Medicine.* 2018;379(8):722-730.
4. Brown PD, Jaeckle K, Ballman KV, et al. Effect of Radiosurgery Alone vs Radiosurgery With Whole Brain Radiation Therapy on Cognitive Function in Patients With 1 to 3 Brain Metastases. *JAMA.* 2016;316(4):401.
5. Hong AM, Fogarty GB, Dolven-Jacobsen K, et al. Adjuvant Whole-Brain Radiation Therapy Compared With Observation After Local Treatment of Melanoma Brain Metastases: A Multicenter, Randomized Phase III Trial. *Journal of Clinical Oncology.* 2019;37(33):3132-3141.
6. Aoyama H, Shirato H, Tago M, et al. Stereotactic Radiosurgery Plus Whole-Brain Radiation Therapy vs Stereotactic Radiosurgery Alone for Treatment of Brain MetastasesA Randomized Controlled Trial. *JAMA.* 2006;295(21):2483-2491.
7. Anderson ES, Postow MA, Wolchok JD, et al. Melanoma brain metastases treated with stereotactic radiosurgery and concurrent pembrolizumab display marked regression; efficacy and safety of combined treatment. *Journal for ImmunoTherapy of Cancer.* 2017;5(1).
8. Chen L, Douglass J, Kleinberg L, et al. Concurrent Immune Checkpoint Inhibitors and Stereotactic Radiosurgery for Brain Metastases in Non-Small Cell Lung Cancer, Melanoma, and Renal Cell Carcinoma. *Int J Radiat Oncol Biol Phys.* 2018;100(4):916-925.
9. Diao K, Bian SX, Routman DM, et al. Combination ipilimumab and radiosurgery for brain metastases: tumor, edema, and adverse radiation effects. *J Neurosurg.* 2018;129(6):1397-1406.
10. Knisely JPS, Yu JB, Flanigan J, Sznol M, Kluger HM, Chiang VLS. Radiosurgery for melanoma brain metastases in the ipilimumab era and the possibility of longer survival. *J Neurosurg.* 2012;117(2):227-233.
11. Liniker E, Menzies AM, Kong BY, et al. Activity and safety of radiotherapy with anti-PD-1 drug therapy in patients with metastatic melanoma. *OncoImmunology.* 2016;5(9):e1214788.
12. Murphy B, Walker J, Bassale S, et al. Concurrent Radiosurgery and Immune Checkpoint Inhibition: Improving Regional Intracranial Control for Patients With Metastatic Melanoma. *American Journal of Clinical Oncology.* 2019;42(3):253-257.
13. Nardin C, Mateus C, Texier M, et al. Tolerance and outcomes of stereotactic radiosurgery combined with anti-programmed cell death-1 (pembrolizumab) for melanoma brain metastases. *Melanoma Research.* 2018;28(2):111-119.
14. Silk AW, Bassetti MF, West BT, Tsien CI, Lao CD. Ipilimumab and radiation therapy for melanoma brain metastases. *Cancer Med.* 2013;2(6):899-906.

15. Tran TT, Mahajan A, Chiang VL, et al. Perilesional edema in brain metastases: potential causes and implications for treatment with immune therapy. *Journal for ImmunoTherapy of Cancer.* 2019;7(1).
16. Pollock PM, Cohen-Solal K, Sood R, et al. Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia. *Nature Genetics.* 2003;34(1):108-112.
17. Wangari-Talbot J, Wall BA, Goydos JS, Chen S. Functional Effects of GRM1 Suppression in Human Melanoma Cells. *Molecular Cancer Research.* 2012;10(11):1440.
18. Isola AL, Eddy K, Zembrzuski K, Goydos JS, Chen S. Exosomes released by metabotropic glutamate receptor 1 (GRM1) expressing melanoma cells increase cell migration and invasiveness. *Oncotarget.* 2017;9(1).
19. Wen Y, Li J, Koo J, et al. Activation of the Glutamate Receptor GRM1 Enhances Angiogenic Signaling to Drive Melanoma Progression. *Cancer Research.* 2014;74(9):2499.
20. Li J, Wen Y, Jeong B-S, et al. GRM1 to induce M-CSF and CCL2 expression to lead to an upregulation of M2 macrophages in melanoma. *Journal of Clinical Oncology.* 2017;35(7_suppl):122-122.
21. Namkoong J, Shin S-S, Lee HJ, et al. Metabotropic Glutamate Receptor 1 and Glutamate Signaling in Human Melanoma. *Cancer Research.* 2007;67(5):2298.
22. Shin S-S, Jeong B-S, Wall BA, et al. Participation of xCT in melanoma cell proliferation in vitro and tumorigenesis in vivo. *Oncogenesis.* 2018;7(11).
23. Yip D, Le MN, Chan JLK, et al. A Phase 0 Trial of Riluzole in Patients with Resectable Stage III and IV Melanoma. *Clinical Cancer Research.* 2009;15(11):3896.
24. Mehnert JM, Silk AW, Lee JH, et al. A phase II trial of riluzole, an antagonist of metabotropic glutamate receptor 1 (GRM1) signaling, in patients with advanced melanoma. *Pigment Cell & Melanoma Research.* 2018;31(4):534-540.
25. Rahma OE, Hodi FS. The Intersection between Tumor Angiogenesis and Immune Suppression. *Clinical Cancer Research.* 2019;25(18):5449-5457.
26. Wallin JJ, Bendell JC, Funke R, et al. Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. *Nature Communications.* 2016;7(1):12624.
27. Makker V, Rasco D, Vogelzang NJ, et al. Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer: an interim analysis of a multicentre, open-label, single-arm, phase 2 trial. *The Lancet Oncology.* 2019;20(5):711-718.
28. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *New England Journal of Medicine.* 2019;380(12):1116-1127.
29. Motzer RJ, Penkov K, Haanen J, et al. Avelumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *New England Journal of Medicine.* 2019;380(12):1103-1115.
30. Silk AW, Saraiya B, Groisberg R, et al. A phase Ib study of troriluzole (BHV-4157) in combination with nivolumab. *J Clin Oncol.* 2020;38:suppl 5; abstr 79.
31. Hodi FS, Chiarion-Sileni V, Gonzalez R, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol.* 2018;19(11):1480-1492.

32. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *New England Journal of Medicine.* 2015;373(1):23-34.
33. Wall BA, Yu LJ, Khan A, Haffty B, Goydos JS, Chen S. Riluzole is a radio-sensitizing agent in an in vivo model of brain metastasis derived from GRM1 expressing human melanoma cells. *Pigment Cell & Melanoma Research.* 2015;28(1):105-109.
34. Beaver JA, Ison G, Pazdur R. Reevaluating Eligibility Criteria — Balancing Patient Protection and Participation in Oncology Trials. *New England Journal of Medicine.* 2017;376(16):1504-1505.
35. Herbst RS, Soria J-C, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature.* 2014;515(7528):563-567.
36. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature.* 2014;515(7528):568-571.
37. Key NS, Khorana AA, Kuderer NM, et al. Venous Thromboembolism Prophylaxis and Treatment in Patients With Cancer: ASCO Clinical Practice Guideline Update. *Journal of Clinical Oncology.* 2019;38(5):496-520.
38. Brahmer JR, Lacchetti C, Schneider BJ, et al. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline. *Journal of Clinical Oncology.* 2018;36(17):1714-1768.
39. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *European Journal of Cancer.* 2009;45(2):228-247.
40. Davies MA, Saiag P, Robert C, et al. Dabrafenib plus trametinib in patients with BRAFV600-mutant melanoma brain metastases (COMBI-MB): a multicentre, multicohort, open-label, phase 2 trial. *The Lancet Oncology.* 2017;18(7):863-873.
41. Qian JM, Mahajan A, Yu JB, et al. Comparing available criteria for measuring brain metastasis response to immunotherapy. *Journal of Neuro-Oncology.* 2017;132(3):479-485.
42. Cai C, Wang S, Lu W, Zhang J. NPHMC: An R-package for estimating sample size of proportional hazards mixture cure model. *Computer Methods and Programs in Biomedicine.* 2014;113(1):290-300.
43. Ivanova A, Qaqish BF, Schell MJ. Continuous Toxicity Monitoring in Phase II Trials in Oncology. *Biometrics.* 2005;61(2):540-545.

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B MULTI-CENTER GUIDELINES

DF/HCC Protocol #: Pending

Dana-Farber/Harvard Cancer Center
Multi-Center Data and Safety Monitoring Plan

1. INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP serves as a reference for any sites external to DF/HCC that are participating in a DF/HCC clinical trial.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Policies and Operations.

2. GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the following general responsibilities apply, in addition to those outlined in DF/HCC Policies for Sponsor-Investigators:

2.1 Coordinating Center

The Coordinating Center is the entity that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines (i.e. CTEP Multi-Center Guidelines).

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain FDA and other correspondence, as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to External Sites as needed.
- Oversee the data collection process from External Sites.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by External Sites and provide to the DF/HCC Sponsor for timely review and submission to the IRB of record, as necessary.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the reporting requirements for the IRB of record to all External Sites.

- Provide External Sites with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor External Sites either by on-site or remote monitoring.
- Maintain Regulatory documents of all External Sites which includes but is not limited to the following: local IRB approvals/notifications from all External Sites, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites
- Conduct regular communications with all External Sites (conference calls, emails, etc) and maintain documentation all relevant communications.

2.1 External Site

An External Site is an institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC investigator. The External Site acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Each External Site is expected to comply with all applicable DF/HCC requirements stated within this Data and Safety Monitoring Plan and/or the protocol document..

The general responsibilities for each External Site may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their IRB of record. For studies under a single IRB, the Coordinating Center will facilitate any study-wide submissions..
- Maintain regulatory files as per ICH GCP and federal requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required.
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities when required by the sponsor.
- Submit Serious Adverse Event (SAE) reports to sponsor, Coordinating Center, and IRB of record as applicable, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to the Sponsor, Coordinating Center, and IRB of record as applicable..
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.

- Notify the sponsor immediately of any regulatory authority inspection of this protocol at the External Site.

3. DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

Certain DF/HCC Policy requirements apply to External Sites participating in DF/HCC research. The following section will clarify DF/HCC requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Revisions and Closures

The External Sites will receive notification of protocol revisions and closures from the Coordinating Center. When under a separate IRB, it is the individual External Site's responsibility to notify its IRB of these revisions.

- Protocol revisions: External Sites will receive written notification of protocol revisions from the Coordinating Center. All protocol revisions should be IRB approved and implemented within a timely manner from receipt of the notification.

- Protocol closures and temporary holds: External Sites will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the External Sites on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.2 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for External Sites. The External Site consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for Investigator-Sponsored Multi-Center Trials. This document will be provided separately to each External Site upon request.

External Sites must send their version of the informed consent document to the Coordinating Center for sponsor review and approval. If the HIPAA authorization is a separate document, please submit to the sponsor for the study record. Once sponsor approval is obtained, the External site may submit to their IRB of record, as applicable. In these cases, the approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each External Site will identify the appropriate members of the study team who will be obtaining consent and signing the consent form for protocols. External Sites must follow the DF/HCC requirement that for all interventional drug, biologic,

or device research, only attending physicians may obtain initial informed consent and any re-consent that requires a full revised consent form.

3.3 IRB Re-Approval

Verification of IRB re-approval for the External Sites is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received for the External Site on or before the anniversary of the previous approval date.

3.4 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned protocol case number be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.5 Participant Registration and Randomization

To register a participant, the following documents should be completed by the participating External Site and faxed (Fax # 617-582-8216) or e-mailed (JohnV_Collantes@dfci.harvard.edu) to the Study Coordinator:

- Copy of brain MRI
- Signed participant consent form
- HIPAA authorization form (if not included in consent form)
- Eligibility Checklist
- External Site Subject Registration Form (Appendix E)

The participating site will then contact the Study Coordinator (617-632-3469 or email JohnV_Collantes@dfci.harvard.edu) to verify eligibility. The Study Coordinator will follow DF/HCC policy (REGIST-101) and register the participant on the protocol. The Study Coordinator will fax or e-mail the participant study number to the participating site. The Study Coordinator will also contact the participating site and confirm registration.

The Coordinating Center will review the submitted documents in order to verify eligibility and consent. To complete the registration process, the Coordinating Center will:

- Register the participant on the study with the DF/HCC Clinical Trial Management System (CTMS).
- Upon receiving confirmation of registration, the Coordinating Center will inform the External Site and provide the study specific participant case number, and, if applicable, assigned treatment and/or dose level.

At the time of registration, the following identifiers are required for all subjects: initials, date of birth, gender, race and ethnicity. Once eligibility has been established and the participant successfully registered, the participant is assigned a unique protocol case number. External Sites should submit all de-identified subsequent communication and documents to the Coordinating Center, using this case number to identify the subject.

Randomization can only occur during normal business hours, Monday through Friday from 8:00 AM to 5:00 PM Eastern Standard Time.

3.6 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the External Site receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and IRB of record must be notified of any violations to this policy.

3.7 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without IRB approval will be permitted. All External Sites are required to fully comply with this requirement. The process for requesting an eligibility exception is defined in DF/HCC policies and institutional policies of other IRBs.

3.8 Data Management

DF/HCC develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. DF/HCC provides a web based training for all eCRF users.

Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

If study forms are not submitted on schedule, the External Sites will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

3.9 Protocol Reporting Requirements

Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor and to the IRB of record.

3.10 Reporting Procedures

Requests to deviate from the protocol require approval from the IRB of record and the sponsor. All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

3.11 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports per DF/HCC requirements, and ensure that all IND Safety Reports are distributed to the External Sites as required by DF/HCC Policy. External Sites will review/submit to the IRB according to their institutional policies and procedures.

4. MONITORING: QUALITY CONTROL

The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

4.1 Ongoing Monitoring of Protocol Compliance

The External Sites may be required to submit participant source documents to the Coordinating Center for monitoring. External Sites may also be subject to on-site monitoring conducted by the Coordinating Center.

The Coordinating Center will implement ongoing monitoring activities to ensure that External Sites are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

4.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at External Sites that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

4.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each External Site. Accrual will be monitored for each External Site by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination.

5. AUDITING: QUALITY ASSURANCE

5.1 DF/HCC Internal Audits

All External Sites are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2-day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

5.2 Audit Notifications

It is the External Site's responsibility to notify the Coordinating Center of all external audits or inspections (e.g., FDA, EMA, NCI) that involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

5.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center, must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the IRB as applicable.

5.4 External Site Performance

The DF/HCC Sponsor and the IRB of record are charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

External Sites that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be put on hold or closed.

APPENDIX E EXTERNAL SITE SUBJECT REGISTRATION FORM

See next page.

External Site Subject Registration Form

For use on investigator-sponsored, multi-center trials only.

DF/HCC Protocol # _____ Enrolling Site _____

SUBJECT INFORMATION

Subject Initials* _____ Date of Birth† _____/_____/_____

Gender†_____ Race†_____ Zip Code† _____

Ethnicity† ☐ Hispanic ☐ Non-Hispanic ☐ Unknown

Diagnosis†_____

ASSIGNMENT

Indicate the arm / cohort / dose level for this subject, if known _____

Date on study / treatment is scheduled to begin_____

ELIGIBILITY

To be completed by local site Screening Staff. By signing below, I confirm that this subject is [☐ **eligible**/ ☐ **ineligible** / ☐ **eligible with exception**].

Signature:	Date:
Printed Name (include credentials):	

Eligibility Exception (Required only if eligibility exception granted for this subject)

OHRS Other Event # for Eligibility Exception	
IRB Approval Date for Eligibility Exception	
Sponsor Approval Date for Eligibility Exception	

To be completed by DF/HCC Enrollment Monitor.

Signature:	Date:
Printed Name:	

* Subject initials are a minimum requirement for registration by DF/HCC.
† Required by NCI's Clinical Trials Reporting Program. See https://www.cancer.gov/about-nci/organization/ccct/ctrp/accrual

APPENDIX F. PILL DIARY

See separate document.