A PHASE 2, HISTORICALLY CONTROLLED STUDY TESTING THE EFFICACY OF TTFIELDS (OPTUNE®) WITH ADJUVANT TEMOZOLOMIDE IN HIGH-RISK WHO GRADE II AND III ASTROCYTOMAS (FORWARD)

UF IRB Protocol Number: 201800600

National Clinical Trial (NCT) Identified Number: NCT03906448

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Study Chair: David Tran, MD, PhD

Coordinating Center: University of Florida

UF Principal Investigator: Ashley Ghiaseddin, MD

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Summary of Changes from Version 2.1 to 2.3:

Affected Section(s)	Summary of Revisions Made	Rationale
5.2	Revised Exclusion Criteria	FDA Reviewer Suggestion
10.3	Addition of contemporaneous cohort explanation	IRB reviewer suggestion
11.1.9.2	Clarification of data de-identification timeline	Survival Follow-up data will be collected for the duration of the study (5 years)

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STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with Good Clinical Practice (GCP), applicable United States (US) Code of Federal Regulations (CFR), state laws and local requirements. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection and GCP Training.

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

1 PROTOCOL SUMMARY

1.1 Synopsis

Title:	A Phase 2, Historically Controlled Study Testing the Efficacy of TTFields (Optune®) with Adjuvant Temozolomide in High-risk WHO Grade II and III Astrocytomas (FORWARD)
Study Description:	Phase 2, multi-institutional, open-label historically controlled study. The hypothesis of this study is that the addition of TTFields treatment to adjuvant temozolomide (TMZ) will significantly increase overall survival (OS) in patients with newly diagnosed Grade II and III astrocytoma with Telomerase Reverse Transcriptase (TERT) promoter mutations only compared to historical controls.
Objectives:	Primary Objective: 1. To determine whether TTFields combined with adjuvant temozolomide increases OS in patients with newly diagnosed Grade II and III astrocytoma with TERT promoter mutation only, compared to historical (dating back to 2016) and contemporaneous controls at the same project sites. Secondary Objectives: 1. To determine whether the combination of TTFields and adjuvant temozolomide is safe in the newly diagnosed high-risk Grade II and III astrocytoma.

	2. To determine whether TTFields combined with adjuvant temozolomide increases progression-free survival (PFS) in patients with newly diagnosed Grade II and III astrocytoma with TERT promoter mutation only, as compared to historical (dating back to 2016) and contemporaneous controls at the same project sites. 3. To evaluate quality of life (QOL) using Karnofsky Performance Status (KPS), Mini-Mental Status Examination (MMSE), and European Organization for Research and Treatment of Cancer Quality of Life Questionnaire - Cancer and Brain (EORTC QLQ-C30/BN20) scale in patients treated with TTFields combined with adjuvant temozolomide, compared to historical (dating back to 2016) and contemporaneous controls at the same project sites. Exploratory Objectives: 1. To determine whether the addition of TTFields to adjuvant temozolomide significantly increases mutation burden, as compared to historical (dating back to 2016) and contemporaneous controls in samples from the same project sites. 2. To determine whether TTFields increases immune cell infiltration. 3. To determine whether TTFields induces immunogenic cell death in tumor.
Endpoints:	Primary Endpoint: Overall survival (OS) Secondary Endpoints: Progression free survival (PFS), safety and quality of life
Study Population:	Patients with tissue-based diagnosis of high-risk Grade II or III astrocytomas, 18 years or older, of both genders after surgery or biopsy followed by radiation therapy with concurrent temozolomide.
Phase:	2
Description of Sites/Facilities Enrolling Participants:	This study is being conducted in a consortium of 10-20 high volume brain tumor centers in the U.S.
Description of Study Intervention:	Treatment with TTFields (Optune®) with Adjuvant Temozolomide: TT Fields are applied through surface transducer arrays placed directly on the shaved head. Temozolomide is available in either tablet or IV infusion.
Study Duration:	60 months: 36 months enrollment plus 24 months follow-up/study completion

Accrual Goal:	100 interventional subjects and up to 100 historical and contemporaneous control subjects
Participant Duration:	24 months (study treatment) plus follow-up until death or until the study is complete/terminates.

1.2 Schema

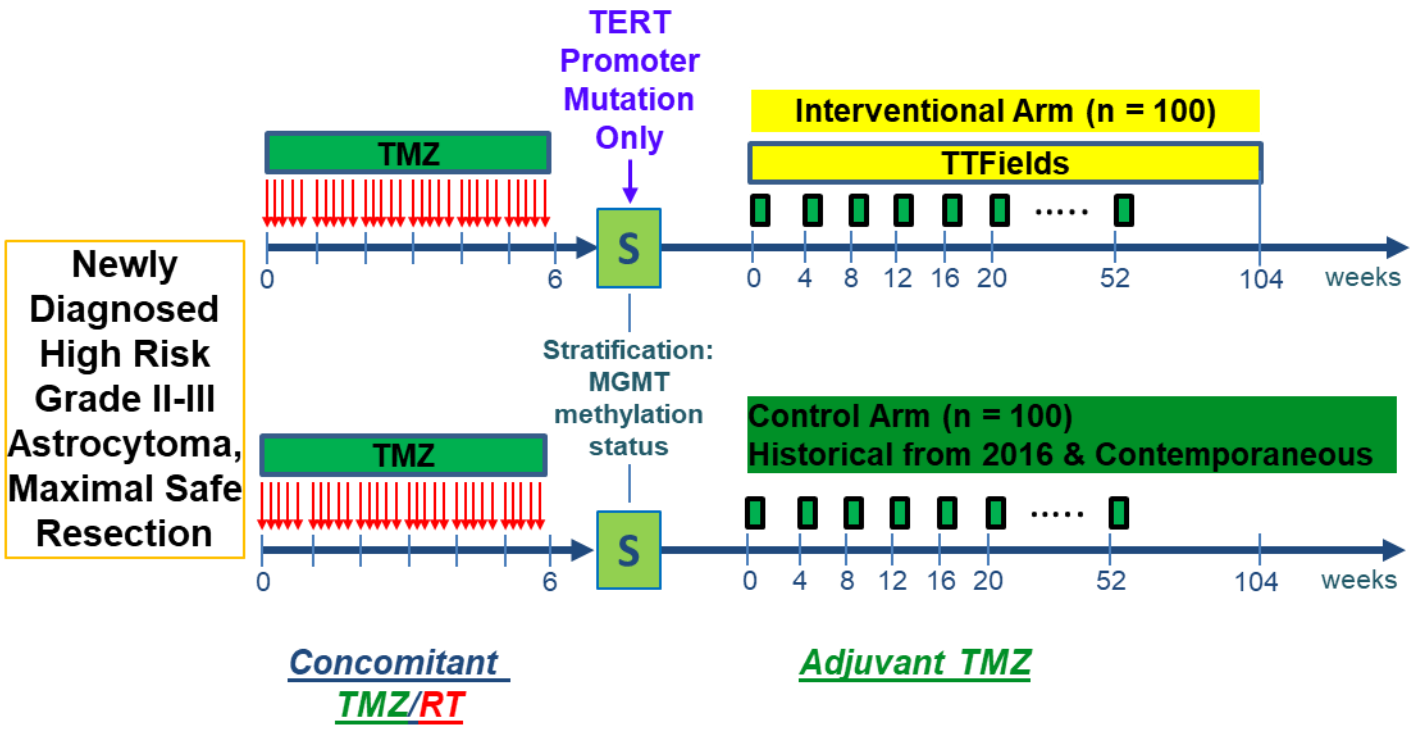

Figure 1. This is a phase 2, Single Arm, Historically Controlled Study of the Combination of Adjuvant Temozolomide plus TTFields in Patients with Newly Diagnosed Grade 2 and 3 Glioma with TERT Promoter Mutation Only The interventional arm will combine TTFields with adjuvant TMZ after the completion of definitive chemoradiotherapy. Study treatment may continue past first tumor recurrence. The primary endpoint will be overall survival. TMZ = Temozolomide; RT = Radiation Therapy.

1.3 Schedule of Activities (SoA)

TABLE 1: SCHEDULE OF ACTIVITIES: INTERVENTIONAL SUBJECTS

Study Procedure:	Screening ≤14 days prior to Day 0	Day 0 : Begin TMZ Treatment	Week 4 (-3/+7 days) and every 4 weeks until treatment stop	Week 8 (±7 days) and every 8 weeks only until treatment stop	Progression	After Treatment Stop (Week 4 (+/- 7 days) from end of treatment)	Survival follow-up
Informed Consent	X						
Medical History, Demographics	X						
Medication History	X						
MGMT methylation status	X						
Tumor Tissue Sample[9]	X				X		
MRI of the brain[1]	X			X	X		
Physical Exam	X		X		X	X	
Neurological status, KPS	X		X		X	X	
CBC w/diff	X[10]		X		X	X	
Pregnancy test (urine or serum)	X[10]						
Biochemistry Panel[6]	X[10]		X		X	X	
Record Steroid Dose	X		X		X		
Quality of Life Questionnaires[2]	X			X	X		
RANO	X			X	X		
Temozolomide Cycle		X[7]	X[8]				

Study Procedure:	Screening ≤14 days prior to Day 0	Day 0 : Begin TMZ Treatment	Week 4 (-3/+7 days) and every 4 weeks until treatment stop	Week 8 (±7 days) and every 8 weeks only until treatment stop	Progression	After Treatment Stop (Week 4 (+/- 7 days) from end of treatment)	Survival follow-up
Optune Therapy[4]		X	X		X		
Optune Compliance[4]			X				
Adverse Events		X	X	X	X	X	
Concomitant Medications		X	X	X	X	X	
Telephone Interview							X[5]
DICOM Images shipped for electric fields mapping[11]		X			X		X

1) Screening MRI should be done within 21 days of study treatment start. MRI of the brain will be performed routinely at baseline and again every 8 weeks +/- 7 days until treatment termination or second progression, whichever is first. An MRI of the head will be obtained within 21 days in the event of clinical signs of progression.
After the post-treatment follow up visit and the patient has not progressed by treatment termination, patient will have contrast-enhanced brain MRI every 3 +/- 1 months for 12 months, then every 6 +/- 2 months for 24 months, then yearly +/- 1 month thereafter or until disease progression, whichever comes first.
All MRI scans should be performed with and without contrast and include the scalp.

2) Quality of Life will be measured using the EORTC QLQ-C30/BN20 and MMSE every 8 weeks +/- 7 days for the first 2 years then every 3 months thereafter.

3) Screening visit should occur within 14 days of adjuvant temozolomide start, with the exception of MRI which can be within 21 days (3 weeks) of adjuvant temozolomide start.

4) Optune therapy should start within two weeks of start of adjuvant temozolomide, but not more than 7 weeks from the end of temozolomide/RT and will be continuous throughout study. Compliance documentation will be obtained every 4 weeks +/- 7 days.

5) Every 3 months until death. The only data required during survival follow-up is DOD.

6) Biochemistry panel will include electrolytes, creatinine, bilirubin, liver enzymes.

7) Patients will begin treatment with temozolomide at 4 weeks (+14 days) from end of radiotherapy. Cycle 1 will be dosed at 150mg/m^2 daily for 5 consecutive days of a 28-day cycle.

Study Procedure:	Screening ≤14 days prior to Day 0	Day 0 : Begin TMZ Treatment	Week 4 (-3/+7 days) and every 4 weeks until treatment stop	Week 8 (±7 days) and every 8 weeks only until treatment stop	Progression	After Treatment Stop (Week 4 (+/- 7 days) from end of treatment)	Survival follow-up
8) Patients will begin treatment with temozolomide Cycle 2 and subsequent cycles every 4 weeks (-3/+7 days) at 200mg/m^2 PO daily for 5 consecutive days, if CTC hematologic toxicity for cycle 1 is grade < 1 and the CTC non-hematologic toxicity for cycle 1 is grade ≤ 2. If dose escalation is not appropriate, the dose should remain at 150 mg/m^2 PO daily for 5 consecutive days for Cycle 2 and onward.							
9) Verification of tissue availability and location should be determined at screening visit. When available, formalin fixed and paraffin embedded (FFPE), pathology slides and/or frozen samples will be obtained for primary tumors and first recurrent tumors.							
10) Labs to be completed within 3 days for pre-TMZ cycles. Screening and other labs are within the visit windows noted. Pregnancy Test required for WOCBP only at Screening.							
11) DICOM images will be shipped to Beth Israel Deaconess Medical Center for electric field mapping analysis at baseline, first progression and second progression. Imaging performed between shipping time points should be included in the next shipment.							

2 INTRODUCTION

2.1 Study Rationale

2.1.1 High-risk Grade II and III Gliomas

A long-standing observation in neuro-oncology concerns a group of Grade II and III gliomas that appear relatively indolent histologically but are highly aggressive and resistant to standard chemoradiation therapy with an overall survival rate similar to that of Grade IV astrocytomas (glioblastoma - GBM). Recent therapeutic advancements in low-risk Grade II and III gliomas have further shined the spotlight on this high-risk group, widely recognized as a major remaining roadblock to achieving long-term survival in all Grade II and III gliomas. In recent years, molecular and genomic reclassification of gliomas has identified this high-risk group of Grade II and III tumors that require special attention as new treatment modalities are developed in the hope of improving its outcomes. Addressing this unmet need is the overarching goal of this protocol.

Common point mutations at the R132 position of the cytosolic NADP+-dependent isocitrate dehydrogenase gene 1 (IDH1) occur in a majority of Grade II and III gliomas (70-80% in oligodendrogliomas and 60-70% in astrocytomas) and secondary GBM (~10%) [1-3] and confer better prognosis and response to therapy. At this position, the R132H mutation accounts for approximately 90% of all IDH1 mutations [1, 3]. Mutations in the related mitochondrial IDH gene IDH2 are much less common and exclusive of IDH1 mutations and occur at the pos ition R172 with the R172K mutation accounting for nearly 65% of all IDH2 mutations [1].

Another important recurrent mutation that occurs exclusively in Grade II and III oligodendrogliomas is the 1p/19q co-deletion [per the new WHO grading system for brain tumors-[4]], which carries both positive prognostic and predictive values [5-7]. By this new classification, astrocytomas lack 1p/19q co-deletion and fare much worse compared to oligodendrogliomas, especially those without IDH mutations. New efforts have been directed at further defining these high-risk subgroups of astrocytomas, including ATRX mutations in alpha-thalassemia/mental retardation syndrome X-linked (ATRX) gene [8-10], which impair non-homologous end joining (NHEJ) repair and are correlated with the alternative lengthening of telomeres (ALT) phenotype, but are mutually exclusive with 1p/19q co-deletion (therefore, for the purpose of this study, the presence of ATRX mutations is considered equivalent to the absence of 1p/19q co-deletion)leading to improved response to cytotoxic treatment, and more recently mutations in the promoter of telomerase reverse transcriptase (TERT), the catalytic subunit of the enzyme telomerase, which, together with the telomerase RNA component (TERC), comprises the most important unit of the telomerase complex. TERT promoter mutation increase telomerase activity and occur in both astrocytomas and oligodendrogliomas of all grades [11, 12].

Until recently, treatment standards for WHO Grade II and III gliomas were poorly defined as few phase 3 clinical trials were available to delineate a role for adjuvant therapy after radiotherapy (RT), which historically had demonstrated a PFS, but not OS, benefit in several studies [13, 14]. In the last five years, however, three large phase 3 studies (two for Grade III and one for Grade II) have established an important role for adjuvant chemotherapy for the standard treatment for these tumors [15-17].

Similar to Grade II oligodendrogliomas, the addition of the chemotherapy regimen PCV to RT in Grade II astrocytomas increased both PFS and OS (Fig. 2A and 2C), although the magnitude of benefit in astrocytomas is significantly less compared to the more favorable oligodendroglioma subtype. As projected, the presence of IDH1 R132H mutation predicted for better response to PCV (Fig. 2B and 2D). However, since there was no breakdown in the fraction of IDH1 R132H mutation in astrocytomas and oligodendrogliomas in this study because the number of tumors lacking a IDH mutation was too few to assess the association with treatment [11], the assumption is that the predictive value of the IDH1 R132H mutation is of equal importance in both of these histologic subtypes. In addition, it is unclear, whether other mutations at positions R132 of IDH1 and R172 of IDH2 carry similar predictive value.

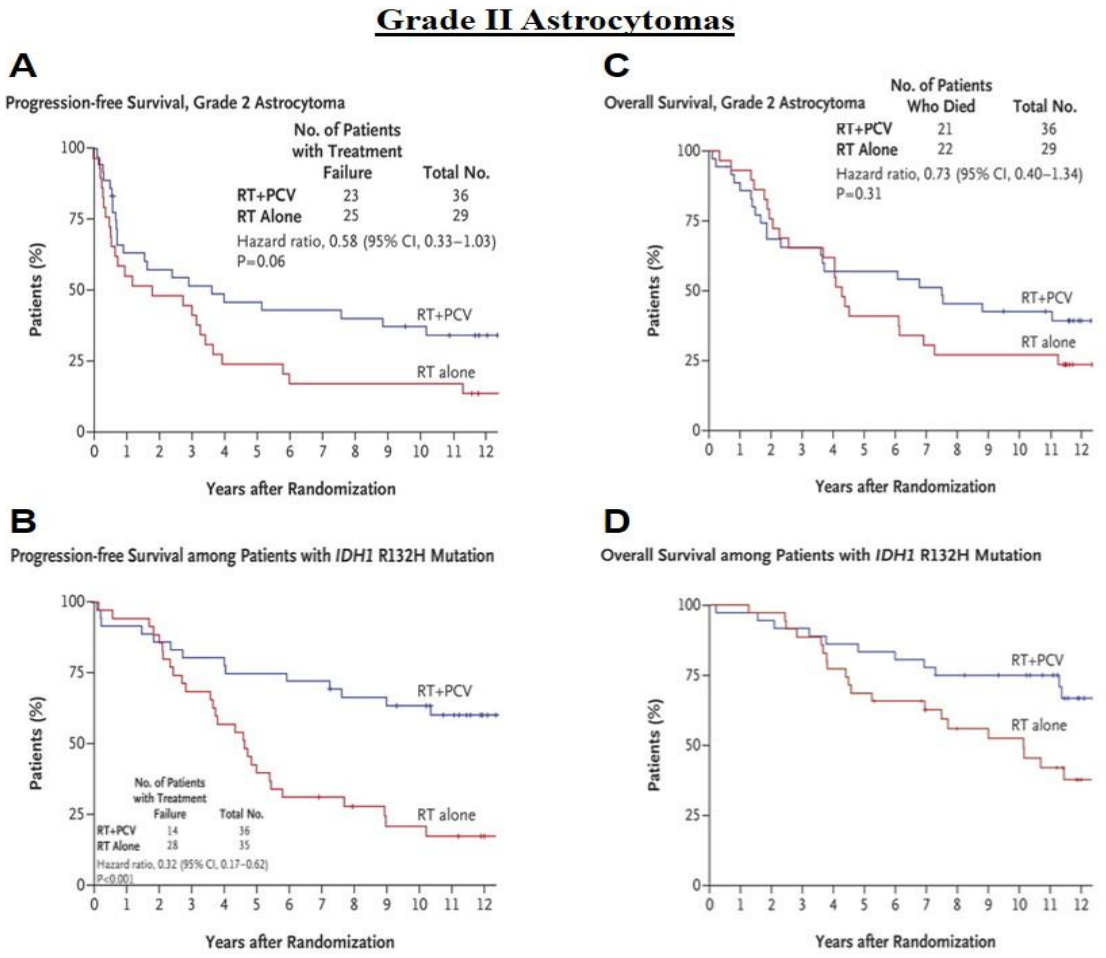

Figure 2. Role of IDH1 R132H Mutation in Grade II Astrocytoma. (A-B) PFS comparison of RT alone (RT) or RT + PCV in Grade II astrocytoma (A) and in all Grade II gliomas (both oliodendrogliomas and astrocytomas) (B) with IDH1 R132H mutation. (C-D) OS comparison of RT alone (RT) or RT + PCV in Grade II astrocytoma (C) and in all Grade II gliomas (both oliodendrogliomas and astrocytomas (D) with IDH1 R132H mutation. (Adapted from Buckner JC et al. N Engl J Med 2016;374:1344-1355).

Of note, information on 1p/19q co-deletions was not available in this study; however, it can be assumed that there would be a strong correlation between the presence of 1p/19q co-deletions and better response to PCV and prognosis compared to those without 1p/19q codeletions, as demonstrated for Grade III gliomas. In summary, Grade II tumors without 1p/19q co-deletions (i.e. astrocytomas per new classification scheme [4]) and IDH mutations fare much worse compared to their counterparts with either or both of these recurrent alterations.

In Grade III gliomas, the predictive roles of 1p/19q co-deletions and IDH mutations, specifically the IDH1 R132H mutation, are better defined thanks to two large phase 3 studies [16, 17]. The presence of 1p/19q co-deletions (i.e. oligodendrogliomas per new classification [4]) significantly increased response to PCV chemotherapy and prolonged survival compared to 1p/19q intact tumors (i.e. astrocytomas) (Fig. 3). The additive presence of the IDH1 R132H mutation further defined 3 distinct groups of tumors with the combined 1p/19q co-deletions and IDH mutations group fairing the best, the double negative the worst, and the single positive group in the intermediate (Fig. 4).

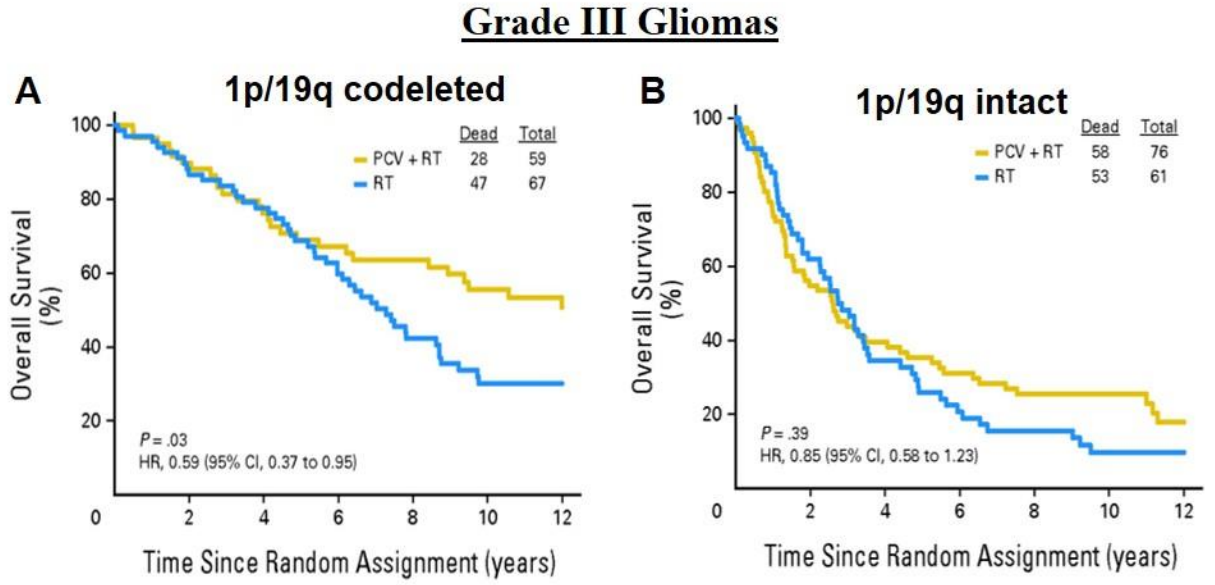

Figure 3. Role of 1p, 19q Codeletions in Grade III Glioma. OS comparison of RT alone (RT) or RT + PCV in Grade III gliomas with 1p, 19q codeletions (A) or without 1p, 19q codeletions (B). (Adapted from Gregory Cairncross et al. JCO 2013; 31:337-343).

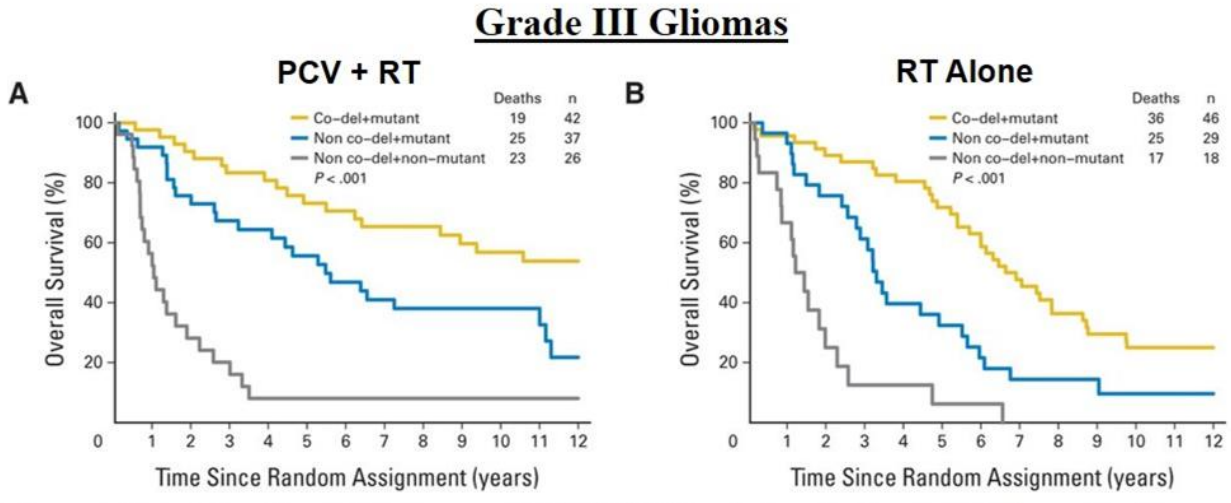

Figure 4. Role of 1p, 19q Codeletions and IDH1 R132H mutations in Grade III Glioma. Kaplan-Meier estimates of OS for patients with a Grade III glioma (orange) or without 1p, 19q codeletions and with (blue) or without 1DH1 R132H mutation after PCV + RT (A) or RT Alone (B). (Adapted from Gregory Cairncross et al. JCO 2013;31:337-343).

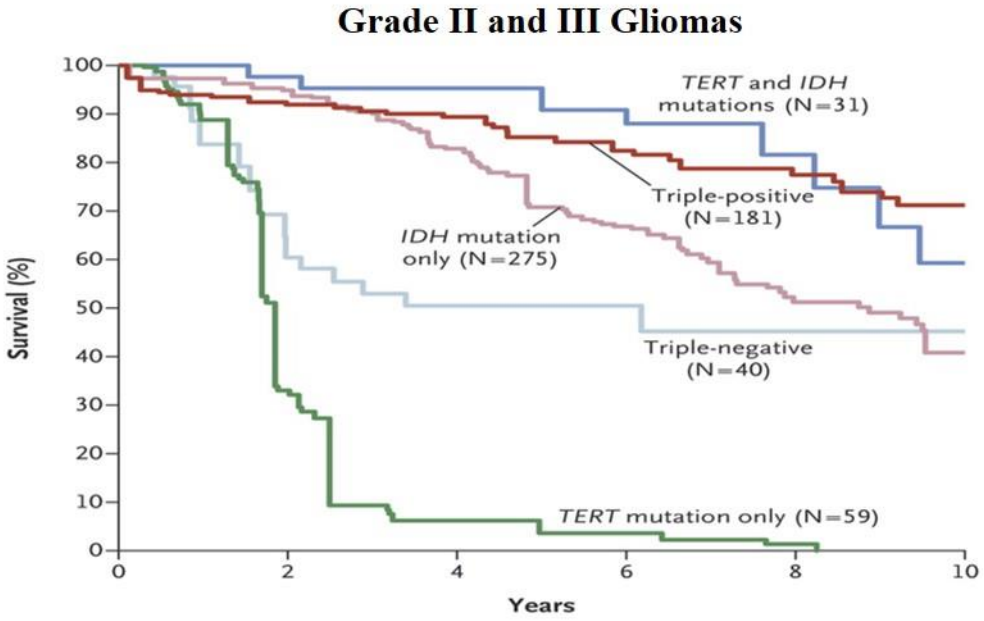

Figure 5. TERT Promoter Mutation only is associated with the worst prognosis in Grade II and III Glioma. The presence of IDH mutations and/or 1p/19q codeletions were able to rescue the poor prognosis associated with TERT promoter mutation. Triple-positive: IDH mutations, 1p/19q codeletions, and TERT promoter mutation; Triple-negative: No IDH mutations, 1p/19q intact, and no TERT promoter mutations. (Adapted from Eckel-Passow JE et al. N Engl J Med 2015;372:2499-2508).

2.1.1.1 Reclassification of WHO grade II and III astrocytomas with high-risk molecular features to molecular GBM

To further delineate and stratify risks within the double negative group of astrocytomas that are resistant to current standard chemoradiation therapy and whose survival approaches that of GBM, Eckel-Passow et al. scored 1087 gliomas, of which 596 were Grade II and III tumors, as negative or positive for the three biomarkers: 1p/19q co-deletions, IDH mutations, and TERT promoter mutations [11]. Using 11,590 controls, association between these genotypic groups and clinical data were examined. As previously demonstrated, TERT promoter mutation occurred in both the most aggressive form of gliomas (GBM) and the best prognostic group (oligodendrogliomas with 1p/19q codeletions and an IDH mutation) (Fig. 5). Importantly, astrocytomas that lacked the 1p/19q co-deletions and IDH mutations, and possessed TERT promoter mutation, exhibited the worst overall survival rate that was not dissimilar to that of GBM, suggesting that these tumors represent a molecular continuum with GBM even though histologically they are Grade II or III.

Beside the Eckel-Passow report [18], a large growing body of literature over the past decade confirms this distinct group of diffuse astrocytomas WHO grade II and III carrying the same molecular high risk features as primary astrocytomas WHO grade IV (i.e. GBM), despite their histologic appearance similar to regular WHO grade II and III tumors. This high-risk group of astrocytomas invariably carry isolated TERT promoter mutations without the presence of IDH mutations [19]. Recently the c-IMPACT-NOW consortium reaffirmed the central role of the poor prognostic indication of diffuse astrocytomas with TERT promoter mutations in the absence of IDH mutations [20], while others support the reclassification of this high-risk diffuse astrocytomas to represent molecular GBM [21], with an aggressive clinical course essentially similar to histologic primary GBM, and treated as regular GBM [3, 18,

21-24]. Although PCV has demonstrated efficacy in combination with radiotherapy in both grade II and III diffuse gliomas, including both regular-risk and high-risk astrocytomas [15-17], TMZ is increasingly being used in place of PCV because of 1) TMZ's proven activity against newly diagnosed GBM [25] and newly diagnosed grade III astrocytomas [26]; 2) TMZ given sequentially before or after RT resulting in similar positive impact on PFS in gliomas, just as PCV [27]; 3) a systematic review of 19 studies, including 2 randomized control trials and 1407 patients, demonstrating that RT/TMZ is at least as efficacious, and in many cases more efficacious, specifically in high-risk Grade II and III astrocytomas as compared to RT/PCV [28]; and 4) the lower toxicity profile and higher ease of administration of TMZ compared to PCV observed in these various trials.

Therefore, these high-risk Grade II and III astrocytomas (1p/19q intact, IDH wild-type, TERT promoter mutated) are increasingly being grouped with primary GBM and managed and treated as aggressively as molecular GBM, and new approved therapies that have shown to be effective against primary GBM (e.g. TMZ and TTFields) or novel experimental approaches designed to treat GBM should be tested in this high-risk group of diffuse astrocytomas.

2.1.1.2 Generalizability of results

Brain and other nervous system cancer is most frequently diagnosed among people ages 55-64 according to the National Cancer Institute's Surveillance, Epidemiology, and End Results Program (SEER). The percent of brain and other nervous system cancer deaths is highest among people aged 65-74, with the median age 58 at diagnosis and 65 at death. Given the impact of brain and nervous system cancer on the general older patient population, results from this investigation will be additive to the cancer knowledge landscape for scientists and investigators going forward.

2.1.2 Background

2.1.2.1 Introduction to Electric Fields

In the laboratory setting and in clinical practice, alternating electric fields show a wide range of effects on living tissues. At very low frequencies (under 1 kHz), alternating electric fields stimulate excitable tissues through membrane depolarization [29]. The transmission of such fields by radiation is insignificant and therefore they are usually applied directly by contact electrodes, though some applications have also used insulated electrodes. Some well-known examples of such effects include nerve, muscle and heart stimulation by alternating electric fields [29, 30]. In addition, low frequency pulsed electric fields have been claimed to stimulate bone growth and accelerate fracture healing [31]. However, as the frequency of the alternating electric field increases above 1 kHz, the stimulatory effect diminishes. Under these conditions although a greater fraction of the fields penetrates the cells, due to the parallel resistor-capacitor nature of all biological membranes, the stimulatory power greatly diminishes as the alternating cell membrane hyper–depolarization cycles are integrated such that the net effect is nulled.

At very high frequencies (e.g. MHz), while the integration becomes even more effective, a completely different biological effect is observed. At these frequencies tissue heating becomes dominant due to dielectric losses. This effect becomes more intense as field intensity or tissue dissipation factor increase [32]. This phenomenon serves as the basis for some commonly used medical treatment modalities including diathermy and radio frequency tumor ablation, which can be applied through insulated electrodes [33].

Intermediate frequency electric fields (i.e., tens of kHz to MHz), alternate too fast for causing nerve-muscle stimulation and involve only minute dielectric losses (heating). Such fields, of low to moderate intensities, are commonly considered to have no biological effect [32]. However, a number of non-thermal effects, of minor biological consequence, have been reported even at low field intensities. These include microscopic particle alignment (i.e., the pearl chain effect [34]) and cell rotation [35, 36]. With pulsed relatively strong electric fields, >

103 V/cm and 100 ms pulse length, reversible pore formation appears in the cell membrane, a phenomenon usually called electroporation [37].

2.1.2.2 Novocure's Tumor Treating Electric Fields (TTFields)

Novocure has shown [38] that when properly tuned, very low intensity, intermediate frequency electric fields (TTFields) stunt the growth of tumor cells. This inhibitory effect was demonstrated in all proliferating cell types tested, whereas, non-proliferating cells and tissues were unaffected. Interestingly, different cell types showed specific intensity and frequency dependences of TTField inhibition. It has been shown that two main processes occur at the cellular level during exposure to TTFields: arrest of proliferation and dividing cell destruction. The damage caused by TTFields to these replicating cells was dependent on the orientation of the division process in relation to the field vectors, indicating that this effect is non-thermal. Indeed, temperature measurements made within culture dishes during treatment and on the skin above treated tumors in-vivo, showed no significant elevation in temperature compared to control cultures/mice. Also, TTFields caused the dividing cells to orient in the direction of the applied field in a manner similar to that described in cultured human corneal epithelial cells exposed to constant electric fields [39]. At the sub-cellular level, it was found that TTFields disrupt the normal polymerization-depolymerization process of microtubules during mitosis. Indeed, the described abnormal mitotic configurations seen after exposure to TTFields are similar to the morphological abnormalities seen in cells treated with agents that interfere directly [40, 41] or indirectly [42-44] with microtubule polymerization (e.g. paclitaxel).

2.1.2.3 The Optune Device

The Optune system (NovoTTF™ Therapy) is a portable battery- operated device, which produces TTFields within the human body by means of surface transducer arrays [45-47]. The TTFields are applied to the patient by means of surface transducer arrays that are electrically insulated, so that resistively coupled electric currents are not delivered to the patient. The transducer arrays, which incorporate a layer of adhesive hydrogel and a layer of hypoallergenic medical tape, are placed on the patient's shaved head. The transducer arrays must be replaced every three to four days and the scalp re-shaved in order to maintain optimal capacitative coupling between the transducer arrays and the patient head. All the treatment parameters are pre-set by Novocure so there are no electrical output adjustments available to the patient. Patients and their caregivers are instructed on proper procedures for changing and recharging depleted service batteries and connecting the device to an external battery pack overnight.

Optune is currently FDA-approved as a single modality treatment for recurrent GBM when both surgical and radiotherapy options have been exhausted. In the pivotal EF-11 trial in recurrent GBM, overall survival (OS) of patients treated with the device was equivalent to those treated with standard chemotherapy alone [46]. Six-month progression-free survival (6PFS) analysis favored the TTFields arm compared to the chemotherapy arm, although not statistically significant. Safety and toxicity profile favored the Optune arm compared to the chemotherapy control arm. No device-specific grade 3 and 4 toxicities were identified for hematologic, gastrointestinal, vascular, renal and respiratory disorders. There were also no increased grade 3 and 4 central nervous system adverse events. The most common device specific adverse event was skin rash due to the transducer arrays. In 2017, Optune was approved in combination with first-line adjuvant temozolomide for newly diagnosed GBM (see below).

2.1.2.4 Effect of Optune on Newly Diagnosed GBM Patients - A Phase 3 Study

Six-hundred ninety-five patients from 83 centers across the world were treated. All patients underwent surgery and radiotherapy for the primary tumor. All patients received temozolomide as adjuvant chemotherapy and were then randomized to standard adjuvant temozolomide chemotherapy (for 6-12 cycles) with or without concomitant administration of TTFields. There was a 2.7- month PFS increase with a median PFS of 6.7 months compared to 4.0 months in the control group (HR, 0.63; 95% CI, 0.52-0.76; $P < .001$) [47, 48]. Median overall survival results increased from 16 months in the temozolomide alone group to 20.9 months in the TTFields plus adjuvant temozolomide group

(HR, 0.63; 95% CI, 0.53-0.76; *P* < .001). [48]Systemic adverse event frequency was 48% vs. 44% in the combination arm vs. temozolomide alone arm, respectively. These results established a new standard treatment for newly diagnosed GBM [47, 48].

2.1.2.5 Temozolomide

Adjuvant temozolomide and radiation therapy following surgery has been shown to improve survival by about 20%. According to the temozolomide package insert adjuvant temozolomide treatment delays disease progression (from 5 to 6.9 months) and improves overall survival (from 12.1 to 14.6 months) in classical GBM histology [25].

2.1.2.6 Correlative Study Background

2.1.2.6.1 THE IMMUNOLOGICAL BASIS BEHIND TTFIELDS

Although immunotherapy, especially immune checkpoint inhibitors (ICIs), has proven to be efficacious against many cancers including lung, head and neck, renal and urothelial cancers, melanoma and lymphoma, their activities against poorly immunogenic cancers such as malignant gliomas remain less defined. Several intratumoral factors have been identified to correlate with responses to ICIs. 1) Mutational burden and landscape have been demonstrated to predict response to ICIs in several cancers, through various mechanisms including correlation between high mutational burden and higher checkpoint ligand expression [49-51], and the observation that effective anti-tumor immune response can be elicited against tumor neoantigens, which are more abundant in tumors with higher mutational burden [52, 53]. Tumors with high mutational burden due to microsatellite instability and/or defective mismatch repair mechanisms have some of the highest response rates to ICIs [54-61]. 2) The presence of tumor infiltrating lymphocytes (TILs), particularly CD8+ TILs, has also been correlated with response to ICIs [62]. TILs may help drive expression of PD-L1 expression in tumors [63]. However, the composition of TILs is emerging as a more reliable predictor of outcomes and response to ICIs. TILs composed mostly of type 1 T cells with high TBET expression and type 1 interferon response are associated with better outcomes, whereas TILs enriched in regulatory T cells expressing the forkhead box transcription factor (Foxp3) (Tregs) confer a significantly poor prognosis and severe resistance to ICIs [64].

Gliomas are generally considered to have low mutation burden compared to ICI-sensitive tumors [65]. The combination of radiotherapy and temozolomide, an alkylating agent, has been suggested to increase mutation burden in gliomas [66], however it remains unclear if this modest increase was sufficient to improve response to ICIs. Early clinical experience of ICIs in recurrent malignant gliomas showed minimal efficacy [67, 68]. However high-grade gliomas with a hypermutant phenotype, such as in patients with germline biallelic mismatch repair deficiency, demonstrated significant and durable response to ICIs [69], indicating that very high mutation burden may improve gliomas' response to ICIs. Recent study in an animal model of lung cancer suggested that TTFields targeted DNA repair mechanisms [70], which potentially could greatly enhance mutation burden in treated tumors over time. These results raise the possibility that the addition of TTFields to chemoradiotherapy in glioma treatment may be sufficient at increasing mutation burden above the threshold of necessary for meaningful and durable response to ICIs. Although immunotherapy and ICIs are not the focus of the current protocol, we propose to determine whether TTFields can indeed increase mutation burden since the results will have significant impact for future trials and treatment development for gliomas in general. Tumor mutational burden will be assessed by standard clinical DNA sequencing on pre-treatment (i.e. at initial surgery) and post-treatment (i.e. first radiographic recurrence while receiving adjuvant temozolomide and TTFields and any subsequent recurrences, where available from standard care procedures) tumor specimens. These results will be compared to historical and concurrent data available on patients with malignant gliomas similarly treated at participating institutions with radiation and temozolomide only and without TTFields. Both microsatellite status and mutation burden, expressed as number of mutations per Mb, will be obtained and compared between the 2 groups.

Radiographic responses to TTFields have been noted to be delayed, in many instances, for >4 months after treatment initiation. In fact, most responders to TTFields experienced transient increases in enhancement, FLAIR and vasogenic edema within 2-4 months of treatment initiation, followed by a gradual decrease in enhancement and edema (EF-11 and EF14 study) [46, 47]. These observations suggest a possibility that TTFields may induce an inflammatory reaction in the tumor. In animal models of cancer, TTFields therapy to the primary tumors induced a considerable increase in peri- and intra-tumoral immune cell infiltration, including both $CD4^+$ and $CD8^+$ lymphocytes, compared to sham-treated control tumors [71]. Although the mechanism remains unclear, it is presumably through its anti-mitotic activities causing immunogenic cell death of cancer cells, leading to an increase in cancer-associated antigen or neoantigen presentation and immune activation, including type 1 interferon responses. In this protocol, we will seek to confirm these observations using patients' tissue samples with an eye toward future combinations of TTFields and immunotherapy.

2.1.2.6.2 THE PHYSICS THAT GOVERNS TTFIELDS

TTFields are lower frequency electromagnetic energies operating at 200 kHz and they are administered externally on the scalp by the Optune® device that consists of two pairs of transducer arrays positioned orthogonally from each other and are connected to a portable electric field generator [72]. The distribution of these electric fields within the intracranial space is governed by the laws of physics, specifically Coulomb's Law, Gauss' Law, Ohm's Law and the principles of continuity and capacitance. Derivatives of these laws also comprise principles such as capacitive reactance and specific absorption rate, which are critical for the characterization of the respective electric field distribution and power absorption rate within the brain [73]. Furthermore, the distribution of TTFields are dependent on tissue characteristics, such as physical density, geometry, electric conductivity and relative permittivity, which in turn determine the changing magnitude and direction of the flow of electric charges flow through different regions of the brain as a function of time. Preclinical experiments have shown that TTFields at 200 kHz interfere with dividing cancer cells as they undergo the metaphase-to-anaphase transition in mitosis with evidence that they may perturb the functions of α/β Tubulin and Septin 2,6,7 protein complex, both of which possess high dipole moments, leading to cellular stress, transcriptional alteration and dysfunctional DNA repair [38, 70, 74]. However, the modeling of TTFields in the brain and therefore treatment verification requires complex computational simulation and modeling of electromagnetic wave propagation, which entails added difficulties due to the different conductivity and relative permittivity values of various intracranial tissues and cavities [73, 75, 76].

In order to visualize the distribution of TTFields in the tumor and the surrounding brain tissues, the coupled Maxwell equations must be solved using numerical approximations such as finite element modeling. These computer modeling procedures involve the segmentation of various brain tissues as seen on patient MRI datasets, the application of appropriate initial and boundary conditions and material properties and finding the solution to the coupled Maxwell equations using finite element analysis. A heatmap of the electric field distribution within the human brain can be derived as well as the electric field-volume and specific absorption ratio-volume histograms can be constructed [73]. From these histograms, the electric field parameters, including E_{AUC}, E_{V150}, $E_{95\%}$ and $E_{50\%}$, as well as specific absorption ratio parameters, including SAR_{AUC}, $V_{SAR7.5}$, $SAR_{95\%}$ and $SAR_{50\%}$, can then be used to compare the dosage applied to different patients. Past effort has shown that the electric field strength estimated by computer modeling was within 10% of that measured directly [45]. However, aggregate analysis is lacking on a larger homogeneous patient population that has been treated by TTFields. Therefore, in this protocol, we will perform computer modeling of electric field and energy deposition in the tumor and then perform correlative analysis with respect to overall survival, progression-free survival and response.

2.1.3 Risk/Benefit Assessment

2.1.3.1 Known Potential Risks

2.1.3.1.1 POTENTIAL ADVERSE EVENTS: OPTUNE

Treatment with the Optune is not expected to cause any serious side effects. However, it is possible that treatment may cause any of the following:

- Local warmth and tingling “electric” sensation beneath the transducer array
- Allergic reaction to the adhesive or the gel
- Skin irritation or skin breakdown
- Infection at the sites of electrode contact with the skin
- Transducer array overheating leading to pain and/or local skin burns
- Headache
- Fatigue

2.1.3.1.2 POTENTIAL ADVERSE EVENTS: TEMOZOLOMIDE

Treatment with temozolomide commonly (>30%) causes the following adverse events:

- Headache
- Fatigue
- Nausea
- Vomiting
- Constipation

Treatment with temozolomide commonly (10-30%) causes the following adverse events:

- back, abdominal and/or stomach pain, breast pain
- diarrhea
- alopecia (hair loss)
- dry skin, skin redness, itching and/or rash,
- erythema multiforme (a skin condition that is similar to a bad rash)
- swelling of extremities (arms, legs, fingers, or toes)
- inflammation or swelling of the mouth, throat and/or sinuses
- confusion, amnesia, dizziness, fever, and/or weakness
- anxiety, depression
- joint and muscle pain
- hemiparesis
- asthenia
- coordination abnormal
- viral infection
- insomnia
- changes in sense of taste
- changes in vision such as double or blurred vision
- coughing or shortness of breath, respiratory tract infection
- urinary incontinence/frequency, urinary tract infection
- weight increase

- seizures, hemiparesis (weakness on one side of the body)
- adrenal hypercorticisim (elevated hormone levels)
- allergic reactions, sometimes severe
- hepatotoxicity
- lymphopenia, thrombocytopenia, neutropenia, and leukopenia
- anorexia
- convulsions
- Very rare side effects have included secondary cancers (including leukemia and myelodysplastic syndrome (MDS))

2.1.3.1.3 POTENTIAL ADVERSE EVENTS: TEMOZOLOMIDE PLUS OPTUNE

In earlier clinical trials using temozolomide plus Optune, common side effects where Optune and temozolomide were used together were:

- low platelet count
- nausea
- constipation
- vomiting
- fatigue
- medical device site reaction
- headache
- seizures
- depression

In earlier clinical trials where Optune and temozolomide were used together, additional side effects were seen.

- seizure and consecutive seizures
- decrease or loss of ability to carry out daily activities
- neurological decline or death

2.1.3.1.4 ADDITIONAL POTENTIAL RISKS

2.1.3.1.4.1 DISEASE PROGRESSION

Adverse events and complications associated with the underlying disease process, which are unlikely but unknown if related to treatment with Optune together with adjuvant temozolomide include the following adverse events:

- Seizure, including Status Epilepticus
- Neurological and functional decline
- Headaches, nausea and/or vomiting
- Death

2.1.3.1.4.2 CEREBRAL EDEMA

Cerebral edema may be secondary to the disease process itself, the surgical procedure, necrosis from previous radiation, or inflammation due to immune infiltration of the brain or destruction of tumor cells. Symptoms may include, but are not limited to, severe headache, confusion, lethargy, unresponsiveness, coma, or focal neurological deficits. Patients will be monitored throughout the course of the study and those with any signs or symptoms of cerebral edema may need their steroid doses increased, treatment with an osmotic diuretic, or surgical decompression. Edema that fails to respond to aggressive therapy may lead to permanent neurological impairment. The probability of this risk can be predicted to some degree based upon tumor size, location and pre-operative neurological impairment. Patients will be monitored throughout the course of the study.

2.1.3.1.4.3 PHLEBOTOMY

Drawing blood or inserting an intravenous catheter into an arm vein may result in bruising or swelling in the area of the insertion, bleeding at the site of the needle puncture, light headedness, fainting and very rarely, local infection, which may be severe.

2.1.3.1.4.4 MRI

The risks and/or discomforts associated with the performance of MRI include the anxiety produced from being in a tight, enclosed space (claustrophobia). In addition, the machine operates using a large and powerful magnet. The magnetism of the machine attracts certain metals: therefore, people with these metals in their bodies (specifically pacemakers, infusion pumps, metal aneurysm clips, metal prostheses, joints, rods or plates) will be excluded from the study. Patients will also be checked to make sure that they do not bring any metal objects into the MRI facility. Dental fillings are less affected by the magnetic fields generated and are therefore permitted. It will be asked that patients let the physicians conducting this study know of any metal in their bodies other than dental fillings.

2.1.3.1.4.5 ALLERGIC REACTIONS TO CONTRAST AGENTS

During the MRI, patients will be given a contrast agent. The agent is given routinely to obtain enhanced MRI scans of the brain. The agent is administered through the vein and requires the placement of an IV catheter. The catheter placement is similar to drawing blood except that the catheter remains in the vein during the time the agent is actively delivered. The risks of a blood draw and insertion of a catheter are similar. There have been a few, rare cases of allergies to the agent used in MRI contrast-enhanced scans. Patients with any known severe allergies to contrast agents will be scanned using CT instead of MRI. Patients with mild allergies (i.e., rash only) will be pretreated with Tylenol and Benadryl prior to injection of the contrast agent.

2.1.3.1.4.6 CONFIDENTIALITY

Participation in research may result in a loss of confidentiality. All data will be coded to protect the patient's identity; however, records will be made available to individuals involved with the study, the clinical staff administering the study, and regulatory representatives such as the IRB, FDA, OHRP and funding agency. Any publications resulting from this study will not use patient identifying data.

2.1.3.2 Known Potential Benefits

Based on previous studies in GBM with TMZ, increased survival is an potential benefit of Optune in this population of patients with high-risk Grade II and III Astrocytoma.

2.1.3.3 Assessment of Potential Risks and Benefits

Optune is currently FDA-approved as a single modality treatment for recurrent GBM when both surgical and radiotherapy options have been exhausted as well as combination with adjuvant temozolomide for newly diagnosed GBM. In the pivotal EF-11 trial in recurrent GBM, overall survival of patients treated with the device was equivalent to those treated with standard chemotherapy alone [72]. Six-month progression-free survival (6PFS) analysis favored the TTFields arm compared to the chemotherapy arm, although not statistically significant. Safety and toxicity profile favored the Optune arm compared to the chemotherapy control arm. No device-specific grade 3 and 4 toxicities were identified for hematologic, gastrointestinal, vascular, renal and respiratory disorders. There were also no increased grade 3 and 4 central nervous system adverse events. The most common device specific adverse event was skin rash due to the transducer arrays.

Up to 100 patients will be exposed to TTFields treatment during the current trial. Considering the minimal toxicity and promising efficacy seen in the previous trials, the small number of patients exposed to this

treatment in the current study and the poor outcome of these patients despite Temozolomide treatment, we conclude that the possible benefits of TTFields treatment drastically exceed its potential risks.

2.1.3.3.1 EF-14 STUDY EXPERIENCE

Six hundred and ninety five patients from 83 centers across the world were treated. All patients underwent surgery and radiotherapy for the primary tumor. All patients received temozolomide as adjuvant chemotherapy and were then randomized to standard adjuvant TMZ chemotherapy (for 6-12 cycles) with or without concomitant administration of TTFields. During a pre-specified interim and futility analysis after 315 randomized patients reached a minimum follow-up of 18 months there was a 3.1-month PFS increase with a median PFS of 7.1 months compared to 4.0 months in the control group [37].

Increased PFS in the TTFields arm subsequently led to the recommendation from the independent data monitoring committee to terminate the trial early for success and allow standard of care patients to cross over to the TTFields group. The percentage of patients alive at 2 years following enrollment was 43% in the TTFields plus temozolomide group compared to 29% in the temozolomide alone group.

Overall survival results increased from 15.6 months in the temozolomide alone group to 20.5 months in the TTFields plus adjuvant temozolomide group establishing a new standard treatment for newly diagnosed GBM [37].

3 OBJECTIVES AND ENDPOINTS

TABLE 2: OBJECTIVES & ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine whether TTFields combined with adjuvant temozolomide increases OS in patients with newly diagnosed Grade II and III astrocytoma with TERT promoter mutation only compared to historical and contemporaneous controls.	OS measured in days from Study Day 0 until death. If the study ends and the participant is still living, OS will be measured in days from Study Day 0.	OS remains the primary concern within this patient population. Intent to Treat analysis will be used to determine if this objective is met.
Secondary		
• To determine whether the combination of TTFields and adjuvant temozolomide is safe in the newly diagnosed high-risk Grade II and III astrocytoma. • To determine whether TTFields combined with adjuvant temozolomide increases PFS in patients with newly diagnosed Grade II and III astrocytoma with TERT promoter mutation only compared to historical and contemporaneous controls. • To evaluate QOL using KPS, EORTC QLQ-C30/BN20, and MMSE in patients treated with TTFields combined with adjuvant temozolomide compared to	• PFS measured in days from Day 0 to progression • Safety measured by incidence and severity of Adverse Events and Toxicities. • Quality of life measured by KPS, EORTC QLQ-C30/BN20, and MMSE • Correlation between % Compliance with TTFields and OS	Safety, Efficacy, and Quality of Life measures for a more complete view of the effects of the study treatment(s).

historical and contemporaneous controls.		
Tertiary/Exploratory		
• To determine whether the addition of TTFields to adjuvant temozolomide significantly increase mutation burden, compared to historical and contemporaneous controls. • To determine whether TTFields increases immune cell infiltration. • To determine whether TTFields induces immunogenic cell death in tumor.	• Mutation burden before and after TTFields, compared to historical and contemporaneous controls • Immune cell infiltration before and after TTFields, compared to historical and contemporaneous controls • Immunogenic cell death marker before and after TTFields, compared to historical and contemporaneous controls • Computer modeling of electric field and energy deposition	To add key immunological and mutational information to the information about these specific tumors.

4 STUDY DESIGN

4.1 Overall Design

4.1.1 Hypothesis

The hypothesis of this study is that addition of TTFields treatment to adjuvant temozolomide will significantly increase OS in patients with newly diagnosed Grade II and III astrocytoma with TERT promoter mutation only compared to historical and contemporaneous controls.

4.1.2 Description

This is a phase 2, multi-institutional, open-label, historically controlled, study of 100 patients with newly diagnosed high-risk Grade II and III astrocytoma combining TTFields with adjuvant temozolomide compared to historical and contemporaneous controls after the completion of definitive chemoradiotherapy. The primary endpoint will be overall survival. This high-risk astrocytoma subgroup constitutes approximately 10% of all Grade II and III gliomas (Fig. 6). Therefore, in order to achieve targeted accrual goals, this study is being conducted in a consortium of 10-20 high volume brain tumor centers in the U.S.

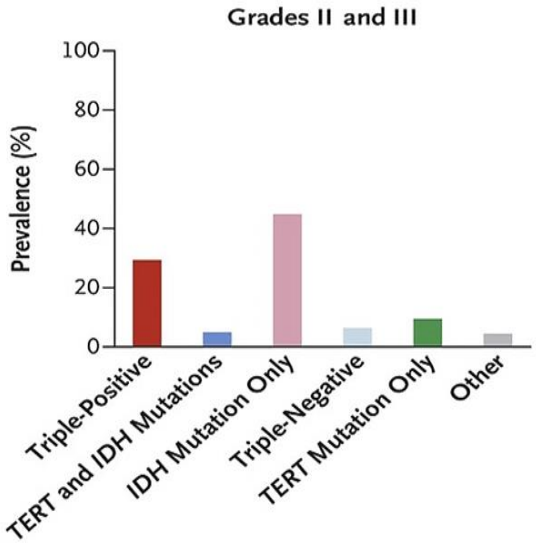

Figure 6 Prevalence of TERT Promoter Mutation in Grade II and III Glioma. (Adapted from Eckel-Passow JE et al. N Engl J Med 2015;372: 2499-2508).

4.1.3 The 2016 World Health Organization (WHO) Classification of Grade II and III gliomas

This new classification provides extensive updates to the previous version from 2007 [77]. For WHO grade II and III gliomas, astrocytomas are now defined by the absence of 1p19q codeletions as determined by FISH, while oligodendrogliomas are defined by the presence of 1p19q codeletions AND IDH1/2 mutation as determined by next generation sequencing (NGS). The concordance rate between 1p19q codeletions and IDH1/2 mutation is extremely high. Gliomas with 1p19q codeletions and without IDH1/2 mutation represents an exceedingly rare entity. In fact, in the Eckel-Passow et al. study, of the 1087 infiltrating gliomas, Pisapia found no case with whole arm 1p19q codeletions was associated with wild-type IDH1/2 [18, 78]. Even when partial arm deletions were included, only 0.3% of cases with partial deletions of 1p19q was without IDH1/2 mutation [18, 78]. In fact, per WHO guidelines, oligodendrogliomas should not be diagnosed in the absence of IDH1/2 mutation (and/or 1p19q codeletions) regardless of histologic appearance since the lack of IDH1/2 mutation is equivalent to the absence of 1p19q codeletions. Thus, the absence of IDH1/2 mutation is mutually exclusive of 1p19q codeletions in the vast majority of cases. Therefore, for the purpose of this study, WHO grade II and III gliomas without IDH1/2 mutation as determined by NGS (rare variants of unknown significance are excluded and not considered to be pathologic mutations) will be considered to also have intact 1p19q (i.e. without codeletions) and a FISH test to determine 1p19q codeletions status will not be required.

4.1.4 Study Sites

A complete list of study sites will be maintained by the Coordinating Center.

4.2 Scientific Rationale for Study Design

See section entitled Study Rationale.

4.3 Justification for Dose

4.3.1 Temozolomide

Dosing instructions are consistent with the usage guidelines for refractory anaplastic astrocytoma listed in the package insert. See package insert for full details.

4.3.2 Optune

Usage instructions are consistent with the usage guidelines approved for Optune by FDA for GBM.

4.4 End of Study Definition

A participant is considered to have completed the study if he or she withdraws consent, is withdrawn by the investigator, is declared lost to follow-up, or dies.

5 STUDY POPULATION

5.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

A. Willing and able to provide written informed consent
B. Stated willingness to comply with all study procedures and availability for the duration of the study
C. Male or female, 18 years or older
D. Life expectancy of at least 3 months
E. Histologic confirmation of WHO Grade II or III astrocytoma
    a. mixed oligoastrocytomas are permitted
F. Mutational identity determined by CLIA-certified sequencing including:
    a. IDH1/2 wildtype (i.e. lack of detectable mutations on the sequencing report, variants of unknown significance or non-synonymous single nucleotide polymorphisms deviating from wild-type sequence do not apply)
    AND
    b. TERT promoter mutation(s)
G. Karnofsky performance status ≥70%
H. Maximal safe resection
    a. biopsy alone is allowed
I. Completed standard chemoradiation with total RT dose of at least 40 Gy and concurrent temozolomide (75mg/m2 daily dose with 80% prescribed dose completed)
    a. Patients with a tumor that was biopsied or resected in the past followed by observation only without definitive chemoradiation and/or chemotherapy given will be eligible, as long as: repeat maximal surgical resection (biopsy only allowed) has been performed, definitive temozolomide/RT treatment meets the criteria above, and adjuvant temozolomide treatment is planned
J. Candidate for adjuvant high dose temozolomide per investigator's clinical judgement
K. Adjuvant Temozolomide start date at least 4 weeks, but not more than 6 weeks, from the later of last dose of concomitant temozolomide or radiotherapy
L. No evidence of early disease progression per RANO criteria at the time of enrollment
M. Women of childbearing potential (WOCBP) must be using a highly effective method of contraception to

avoid pregnancy throughout the study and for at least 24 weeks after the last dose of study drug to minimize the risk of pregnancy.

a. Prior to study enrollment, women of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy.
b. Refer to section 10.2.1 for guidance on highly effective contraceptive methods acceptable in this study.
c. WOCBP include any woman who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who is not post-menopausal. Post-menopause is defined as:
    i. Amenorrhea that has lasted for ≥ 12 consecutive months without another cause, or
    ii. For women with irregular menstrual periods who are taking hormone replacement therapy (HRT), a documented serum follicle-stimulating hormone (FSH) level of greater than 35 mIU/mL.

N. Males with female partners of child-bearing potential must agree to use physician-approved contraceptive methods (e.g., abstinence, condoms, vasectomy) throughout the study and should avoid conceiving children for 24 weeks following the last dose of study drug.
O. Patient must agree to wear the Optune device. If patient declines to wear the Optune device for personal and non-medical reasons (except for history of known allergy to hydrogel) and the plan is to treat the patient with adjuvant TMZ alone, the patient can be considered for enrollment in the contemporaneous control cohort (See Section 9, Control Cohort).

5.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

A. Prior treatment with anti-angiogenic agents including bevacizumab.
B. Prior treatment with TTFields.
C. Progressive disease (according to RANO criteria) after temozolomide/RT.
D. Actively participating in another clinical treatment trial intended to treat the underlying astrocytoma.
E. Females who are pregnant or breastfeeding.
F. Significant co-morbidities at baseline (within 2 weeks prior to adjuvant temozolomide start) which would prevent adjuvant temozolomide treatment:
    a. Thrombocytopenia (platelet count < 100 x 10^3/µL)
    b. Neutropenia (absolute neutrophil count < 1.5 x 10^3/µL)
    c. CTC grade 4 non-hematological toxicity (except for alopecia, nausea, vomiting)
    d. Significant liver function impairment - AST or ALT > 5 times the upper limit of normal
    e. Total bilirubin > 2 times upper limit of normal
    f. Significant renal impairment (GFR ≤ 30 ml/min)
G. Implanted pacemaker, programmable shunts, defibrillator, deep brain stimulator, other implanted electronic devices in the brain, or documented clinically significant arrhythmias.
H. A skull defect such as missing bone with no replacement
I. Bullet fragments embedded the skull
J. Tumors located in the brain stem and/or the cerebellum.
K. History of hypersensitivity reaction to temozolomide, Dacarbazine (DTIC) or hydrogel.

5.3 Additional Considerations

- Operable patients (biopsy alone allowed) must undergo surgery prior to enrollment.
- All patients must have received radiation therapy with concomitant temozolomide prior to enrollment.
- Patients receiving steroids to control edema may be included in the trial; however, any change in steroid dose must be documented during the follow- up visits.
- Disease status will be determined by comparing screening MRI to the immediate post-surgical MRI. If unavailable, an immediate post-surgical CT can be used for the same purpose.
- Patients who have progressive disease at screening (compared to the immediate post-surgical MRI) are not candidates for this study.
- In the case of local radiological suspicion of pseudoprogression, the patient is still eligible for the study per RANO criteria. However, other imaging modalities (e.g. brain PET, perfusion in addition to T1 weighted MRI) can be obtained to assess biological activity of the tumor if desired but not required by the study.

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but do not meet the criteria for participation in this trial. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details and eligibility criteria.

Individuals who do not meet the criteria for participation in this trial (screen failure) may not be rescreened. Participants may repeat individual lab tests within the screening window, but no modifications to the screening window or I/E criteria are permitted. No protocol exceptions are permitted and no waivers will be granted for enrollment into this protocol.

This protocol estimates a 10% screen failure rate.

5.5 Strategies for Recruitment and Retention

Potentially eligible patients scheduled to undergo maximal surgical resection of a Grade II or III astrocytoma will be identified through direct referral at participant sites. PHI will be reviewed by the PI(s) and/or Study Coordinator but not recorded in order to pre-screen patients for eligibility for this trial. The pre-screening PHI will consist of a review of the medical records to confirm new diagnosis in adults age 18 years or older, as well as tumor cytogenetic and DNA sequencing characteristics (Mutational identity determined by CLIA-certified sequencing as possessing a IDH1/2 wildtype and TERT promoter mutated genotype).

Since high-risk astrocytomas are only approximately 10% of all Grade II and III gliomas, only those patients who have appropriate tumor characteristics after biopsy will be approached about the study. Potential subjects will be approached well in advance of study commencement to ensure sufficient time for review of the informed consent form and other logistical coordination.

Since the primary outcome of the study is overall survival, each patient's Legally Authorized Representative (LAR) will be recorded at enrollment. In the event that the subject loses capacity to consent for himself/herself during the course of the study, the LAR will be approached for the Survival Follow-up items (only) for that subject.

Information on this study will be posted on ClinicalTrials.gov. Recruitment is planned for 36 months in total.

5.5.1 Screening

- Patients with newly diagnosed high-risk Grade II or II astrocytoma must undergo maximal safe resection (biopsy alone may be eligible).
- Patients must undergo chemoradiotherapy: concomitant 75mg/m2 daily temozolomide with 80% prescribed dose completed and RT with minimal RT dose of 40 Gy delivered.
- Within three weeks prior to beginning adjuvant temozolomide, all patients will undergo a Baseline contrast-enhanced MRI of the brain.
- Within two weeks prior to beginning adjuvant temozolomide, all patients will undergo the following assessments:
    - Medical History and demographics
    - Medication History
    - Steroid Dose (recorded)
    - MGMT methylation status (if feasible, required for stratification)
    - Tumor Tissue sample – verify availability of FFPE, pathology slides, and/or frozen sample(s)
    - RANO assessment
    - Complete physical examination
    - Neurological status and KPS
    - CBC with differential
    - Pregnancy test (urine or serum) – WOCBP only
    - Biochemistry panel (Electrolytes, creatinine, bilirubin, liver enzymes)
    - Quality of life questionnaires (EORTC QLQ-C30/BN20, and MMSE)

No study-specific procedures may be performed prior to obtaining informed consent. However, assessments performed according to standard of care, prior to consent, may be used to fulfill the screening requirement if they are part of the patient's medical record within the specified timeframe.

See Section "Enrollment Documentation Requirements" for a complete list of patient enrollment documentation.

5.5.1.1 Mini-Mental Status Exam

The Mini-Mental Status Exam is a standardized tool to measure neurocognitive function. The MMSE is scored on a scale of 0 to 30. Throughout this protocol, a cutoff score of 23 or less shall prompt study staff to report the score to the treating physician for clinical decision-making.

5.5.2 Registration

Per UFHCC policy, patients must be registered into UF's OnCore within one business day of consent. Status changes must also be updated within one business day.

Sites should notify the Coordinating Center by one of the following:

- Enter into eCRF (REDCap)
- Email to *Forward-study@ufl.edu*
- Fax to (352) 392-8413 with phone confirmation to (352) 294-8836

A minimum amount of data is required for registration and status changes. CRFs will be provided to all sites via the eCRF.

6 STUDY INTERVENTION

6.1 Study Intervention(s) Administration

6.1.1 Study Intervention Description

6.1.1.1 Treatment Plans – Optune (TTFIELDS) + temozolomide

Patients will begin study treatment with temozolomide and TTFields within 2 weeks of the baseline evaluation, and no later than 6 weeks from last dose of concomitant temozolomide or radiation therapy (the latter of the two). A minimum of 6 and a maximum of 12 cycles of adjuvant temozolomide will be given, depending on tolerability and toxicity.

6.1.1.2 Periodic Evaluation until Treatment Termination

Patients will be seen and examined before each cycle of temozolomide. After a maximum of 12 cycles of adjuvant temozolomide, patients will be seen every 8 weeks. Brain MRI and QoL assessments will be performed every 8 weeks following the baseline MRI for the first 2 years then every 3 months thereafter until second progression (when TTFields treatment will be terminated).

Patients will undergo the following studies or review every month until treatment termination:

- Physical examination
- Neurological status and performance status (KPS)
- Blood exams
- Optune Compliance
- Record of Adverse Events, concomitant medications and steroid dose

Patients will undergo the following studies or review every 8 weeks +/- 7 days until treatment termination:

- Quality of Life Questionnaires (EORTC QLQ-C30/BN20, and MMSE)
- Contrast-enhanced MRI

Screening MRI should be done within 21 days of study treatment start. MRI of the brain will be performed routinely at baseline and again every 8 weeks +/- 7 days until treatment termination or second progression, whichever is first. In case of clinical progression, an MRI will be performed within 21 days.

All MRI scans should be performed with and without contrast.

The data collection for patients who remain on Optune (on-study treatment) and begin 2nd line therapy should include labs and visits adjusted as appropriate for the standard care of that patiet's 2nd line therapy.

6.1.1.3 Disease Progression

The following will be considered disease progression for determination of PFS using the updated response assessment criteria for high-grade gliomas: Response Assessment in Neuro-Oncology (RANO) working group guideline [79].

This study will apply the RANO criteria for high grade gliomas to assess radiographic disease status. These criteria specify assessment of enhancing disease to define progression and response. It is likely that a significant number of subjects eligible for the proposed study will have non-enhancing disease. For subjects without enhancing disease to be eligible for the study, the RANO allowance for FLAIR will be utilized for non-enhancing disease. FLAIR considerations are detailed in the tables below.

6.1.1.3.1 CRITERIA FOR DETERMINING FIRST PROGRESSION DEPENDING ON TIME FROM INITIAL CHEMORADIOTHERAPY

TABLE 3. RANO CRITERIA FOR DETERMINING FIRST PROGRESSION

First Progression	Definition
Progressive disease <12 weeks after completion of chemoradiotherapy	Progression can only be defined using diagnostic imaging if there is new enhancement outside of the radiation field (beyond the high-dose region or 80% isodose line) or if there is unequivocal evidence of viable tumor on histopathologic sampling (e.g., solid tumor areas [i.e., > 70% tumor cell nuclei in areas], high or progressive increase in MIB-1 proliferation index compared with prior biopsy, or evidence for histologic progression or increased anaplasia in tumor). Note: Given the difficulty of differentiating true progression from pseudoprogression, clinical decline alone, in the absence of radiographic or histologic confirmation of progression, will not be sufficient for definition of progressive disease in the first 12 weeks after completion of concurrent chemoradiotherapy.
Progressive disease ≥ 12 weeks after chemoradiotherapy completion	1. New contrast-enhancing lesion outside of radiation field on decreasing, stable, or increasing doses of corticosteroids. 2. Increase by ≥ 25% in the sum of the products of perpendicular diameters between the first post-radiotherapy scan, or a subsequent scan with smaller tumor size, and the scan at 12 weeks or later on stable or increasing doses of corticosteroids. 3. Clinical deterioration not attributable to concurrent medication or comorbid conditions is sufficient to declare progression on current treatment but not for entry onto a clinical trial for recurrence. 4. For patients receiving antiangiogenic therapy, significant increase in T2/FLAIR non-enhancing lesion may also be considered progressive disease. The increased T2/FLAIR must have occurred with the patient on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy and not be a result of comorbid events (e.g., effects of radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects).

6.1.1.3.1.1 CRITERIA FOR RESPONSE ASSESSMENT INCORPORATING MRI AND CLINICAL FACTORS

Criteria for Response Assessment Incorporating MRI and Clinical Factors (Adapted from JCO 2010)

TABLE 4. RESPONSE EVALUATION AFTER A COMPLETE RESECTION

Response	Criteria
Complete response	• Requires all of the following: complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. • No new lesions; stable or improved non-enhancing (T2/FLAIR) lesions. • Patients must be off corticosteroids (or on physiologic replacement doses only) and stable or improved clinically. Note: Patients with non-measurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: • ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. • No progression of non-measurable disease. • Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan. • Stable or improved clinically. Note: Patients with non-measurable disease only cannot have a partial response; the best response possible is stable disease.
Stable disease	Requires all of the following: • Does not qualify for complete response, partial response, or progression. • Stable non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.
Progression	Defined by any of the following: • ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids*. The absolute increase in any dimension must be at least 5mm when calculating the products. • Significant increase in T2/FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). • Any new measureable lesion. • Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose. • Failure to return for evaluation as a result of death or deteriorating condition; or clear progression of non-measurable disease.

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

- Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery.
- Stable doses of corticosteroids include patients not on corticosteroids.

The determination of whether to stop treatment due to progression will be based on the investigator's evaluation of the patient's clinical condition. TTFields treatment will be continued for 24 months or until second progression whichever occurs first unless the patient's clinical condition prohibits this. In the case of radiological progression based on local evaluation, temozolomide treatment will be stopped and a second line treatment chosen instead. The following treatments or any combination of these treatments are considered second line therapy in this protocol:

- Re-operation
- Local radiation therapy
- Second line therapy
- Combination of the above

6.1.1.3.1.2 DISEASE PARAMETERS

Measurable disease: Bi-dimensionally measurable lesions with clearly defined margins by MRI scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-measurable or evaluable disease: Uni-dimensionally measurable lesions or lesions with margins not clearly defined such as areas of T2/FLAIR signal abnormality or poorly defined enhancing abnormality.

Note: For cystic lesions, the only measurable part is any enhancement area around the cyst that is clearly defined and bi-dimensionally measurable. The cyst itself should not be considered measurable or non-measureable disease.

Target lesions: All measurable lesions that are seen at baseline MRI. Target lesions should be selected on the basis of their size (lesions with the longest diameter), 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. When there are too many measurable lesions, choose the largest 3 lesions as target lesions to follow. The other measurable lesions should be considered evaluable for the purpose of objective status determination.

Non-target lesions: All non-measurable 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.

6.1.1.3.1.3 METHODS FOR EVALUATION OF MEASURABLE DISEASE

All measurements should be taken and recorded in metric notation using a ruler. All baseline MRI evaluations should be performed as closely as possible to Study Day 0. An MRI should be collected and evaluated after any surgical procedure (surgical resection/debulking) but not biopsy.

Clinical lesions: Clinical lesions will only be considered measurable on brain MRI when they are $\geq$ 10 mm (1 cm) in 2 perpendicular measurements in each of 2 perpendicular planes (i.e., axial and coronal) as assessed using a ruler.

Histology: This technique can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases when biopsy or surgical resection of a measureable lesion is clinically indicated.

Perfusion/CBV: This advanced brain MRI technique can be used as an adjunct test to determine treatment response or disease status. However, it should not be used as the primary or sole method to determine response or disease status.

Brain FDG-PET coupled with head CT or brain MRI and brain spectroscopy: These advanced metabolic imaging techniques can be used as an adjunct test to determine response or disease status. However, they should not be used as the primary or sole method of determining response or disease status.

6.1.1.3.1.4 EVALUATION OF TARGET LESIONS

Complete Response (CR): Disappearance of all target lesions.

Partial Response (PR): ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all target lesions sustained for at least 4 weeks.

Progressive Disease (PD): At least a 25% increase in the sum of products of perpendicular diameters of at least 1 target lesion, taking as reference the smallest sum of products of perpendicular diameters on study (this includes the baseline sum if that is the smallest on study). The absolute increase in any dimension must be at least 5mm when calculating the products of perpendicular diameters.

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

6.1.1.3.1.5 EVALUATION OF NON-TARGET LESIONS

Complete Response (CR): Disappearance of all non-target lesions.

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 on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy* not caused by comorbid events (e.g. Radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). 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 Study Chair.

6.1.1.3.1.6 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.

TABLE 5. SUMMARY OF THE RANO RESPONSE CRITERIA (ADAPTED FROM JCO 2010)

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.

* Progression occurs when this criterion is present.

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

NOTE: Patients may continue on treatment and remain under close observation and evaluation at 4-8 week intervals if there is uncertainty regarding whether pseudoprogression may be present as determined by the investigators. If subsequent radiographic or clinical assessments suggest that the patient is in fact experiencing progression, then the date of progression should be the time point at which this issue was first raised. Similarly, stable disease may be assigned in cases of presumed "pseudoprogression" associated with decreased steroid use.

6.1.1.3.1.7 DURATION OF RESPONSE

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

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

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

6.1.1.3.1.8 NEUROLOGICAL EXAM AND PERFORMANCE STATUS

Patients will be graded using the Karnofsky Performance Status scale and their neurological function evaluated as improved, stable or deteriorated in addition to objective measurement of tumor size. These parameters will be used to deter mine the overall response assessment.

The *Karnofsky Scale* has been adapted for use in many areas, including hospices, cancer clinics, etc., as well as used by various CFS researchers and physicians (Leonard Jason, PhD; Jay A. Goldstein, MD).

The 10-point scale is a quick and easy way to indicate how you are feeling on a given day, without going through several multiple-choice questions or symptom surveys.

TABLE 6: KARNOFSKY SCALE

100	Able to work. Normal; No complaints; No evidence of disease.
90	Able to work. Able to carry on normal activity; Minor symptoms.
80	Able to work. Normal activity with effort; Some symptoms.
70	Independent; not able to work. Cares for self; Unable to carry on normal activity.
60	Disabled; dependent. Requires occasional assistance; cares for most needs.
50	Moderately disabled; dependent. Requires considerable assistance and frequent care.

40	Severely disabled; dependent. Requires special care and assistance.
30	Severely disabled. Hospitalized, death not imminent.
20	Very sick. Active supportive treatment needed.
10	Moribund. Fatal processes are rapidly progressing.

6.1.1.3.1.9 PROGRESSION-FREE SURVIVAL

PFS is defined as the duration of time from start of adjuvant treatment (TMZ C1D1) to time of progression or death, whichever occurs first.

6.1.1.3.2 RESPONSE EVALUATION AFTER A COMPLETE RESECTION

A complete resection will be defined as contiguous contrast-enhancement <10 mm in 2 perpendicular measurements in each of 2 perpendicular planes (i.e., axial and coronal). Because neither the RECIST[80] nor MacDonald [81] criteria are completely appropriate for patients with minimal residual disease, a modified version of these criteria will be used as outlined below.

6.1.1.3.2.1 COMPLETE RESPONSE

Complete disappearance of enhancing tumor on consecutive CT or MRI images at least 1 month apart, and neurologically stable or improved and at the same or a lower steroid dose. To be assigned a status of "Complete Response" as a best overall response, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response is first met.

6.1.1.3.2.2 PARTIAL RESPONSE

Partial response is not assessable in patients without sufficient measurable disease.

6.1.1.3.2.3 STABLE DISEASE

Insufficient change to qualify for Complete Response or Progressive disease.

6.1.1.3.2.4 PROGRESSIVE DISEASE

At least a 25% increase in the longest diameter on an axial image of any enhancing tumor on consecutive CT or MRI images with a minimum of ≥ 5mm of contiguous contrast-enhancement in 2 perpendicular measurements in each of 2 perpendicular planes (i.e., axial and coronal).

6.1.1.3.2.5 NOT ASSESSABLE

Progression has not been documented and one or more sites have not been assessed.

6.1.1.3.2.6 POST-TREATMENT EVALUATION

After treatment termination the patient will be seen at an outpatient clinic for one additional visit within 30 days +/- 7 days. Physical and neurological examination, blood tests (CBC and Biochemistry panel) will be performed during this visit. Patient mortality and adverse events will be documented on the case report forms.

After the post treatment follow up visits, if the patient has not progressed by treatment termination, patient will have contrast-enhanced brain MRI every 3 +/- 1 months for 12 months. Then MRIs every 6 +/- 2 months for 24 months, then yearly +/- 1 month thereafter or until disease progression, whichever comes first.

In case of clinical progression an MRI will be performed within 21 days. After the end of study therapy visit post disease progression, the patient will not be required to return to the clinic for follow-up but will be followed until death by telephone every 3 months to monitor their status.

6.1.2 Dosing and Administration

6.1.2.1 Adjuvant Temozolomide Treatment

All patients will receive adjuvant temozolomide.

6.1.2.2 Adjuvant Phase Cycle 1:

Four to six weeks after completing the temozolomide + RT phase, the temozolomide is administered for an additional 12 cycles of adjuvant treatment. Dosage in Cycle 1 (adjuvant) is 150 mg/m^2 once daily for 5 days followed by 23 days without treatment.

6.1.2.3 Adjuvant Cycles 2-12:

At the start of Cycle 2, the dose is escalated to 200 mg/m^2, if the CTC non- hematologic toxicity for Cycle 1 is Grade ≤2 (except for alopecia, nausea and vomiting), absolute neutrophil count (ANC) is ≥ 1.5 x 10^9/L, and the platelet count is ≥ 100 x 10^9/L.

The dose remains at 200 mg/m^2 per day for the first 5 days of each subsequent cycle unlessunless toxicity occurs. If the dose was not escalated at Cycle 2, escalation should not be done in subsequent cycles.

6.1.2.4 Temozolomide Dose Reduction or Discontinuation

Dose reductions during the adjuvant phase should be applied according to Tables 7 and 8 below. Cycle 1 Day 1 should not start until the ANC is above 1.5 x 10^9/L (1,500/µL) and the platelet count exceeds 100 x 10^9/L (100,000/µL). If unable to start within the Cycle 1 start window, the patient will be considered a screen fail.

The next cycle of temozolomide should not be started until the ANC and platelet count exceed these levels. Dose reductions during the next cycle should be based on the lowest blood counts and worst non-hematologic toxicity during the previous cycle. Dose reductions or discontinuations during the adjuvant phase should be applied according to Tables 7 and 8. Routine blood monitoring should be conducted in accordance with local site procedures.

TABLE 7. TEMOZOLOMIDE DOSE LEVELS FOR ADJUVANT TREATMENT

Dose Level	Dose (mg/m^2/day)	Remarks
–1	100	Reduction for prior toxicity
0	150	Dose during Cycle 1
1	200	Dose during Cycles 2-12 in absence of toxicity

TABLE 8. TEMOZOLOMIDE DOSE REDUCTION OR DISCONTINUATION DURING ADJUVANT TREATMENT

Toxicity	Reduce temozolomide by 1 Dose Level[a]	Discontinue temozolomide
Absolute Neutrophil Count	<1.0 x 10^9/L	See footnote b
Platelet Count	<50 x 10^9/L	See footnote b
CTC Non-hematological Toxicity (except for alopecia, nausea, vomiting)	CTC Grade 3	See footnote b

[a]: temozolomide dose levels are listed in table 7.

[b]: temozolomide is to be discontinued if dose reduction to <100 mg/m^2 is required or if the same Grade 3 non-hematological toxicity (except for alopecia, nausea, vomiting) recurs after dose reduction.

CTC = Common Toxicity Criteria.

6.1.2.5 Treatment after temozolomide discontinuation

Following temozolomide discontinuation due to toxicity or radiological disease progression (based on local interpretation of MRI), patients will be offered second line therapy for their disease. The following treatment options may be considered based on the patient's clinical condition per the treating physician's discretion:

1. Re-operation (TTFields treatment will be interrupted for at least 2 weeks after reoperation or until wound healing is sufficient to resume).

2. Local radiation therapy
3. Second line therapy (at the discretion of the treating physician and not limited to traditional cytotoxic chemotherapy)
4. Combination of the above

TTF treatment will continue concomitant with second line treatments until second disease progression for TTFields + temozolomide patients.

6.1.2.6 Optune (TTFields) + temozolomide

Optune treatment will be initiated in an outpatient clinic by the investigator's team at each center or at home by Novocure's device support specialists (DSS). 3D mapping of tumors using the FDA-approved NovoTAL software will be completed by a NovoTAl-certified treating physician or by Novocure. Scalp shaving, array placement and all device support services (including patients' and families' education of device operation, downloading and processing of technical and compliance data, and troubleshooting device malfunction) will be provided by the DSS for the first placement. Patients and/or their caregivers will continue treatment at home after being trained in device use with the support of DSS as needed.

TTFields will start within 2 weeks of the first cycle of adjuvant temozolomide but must be within 7 weeks after end of temozolomide/RT. Adjuvant Temozolomide will continue for a minimum of 6 and a maximum of 12 cycles. Optume will continue for a maximum of 2 years. At first progression, patients will be allowed to continue with TTFields therapy with any other therapy per standard of care. Patients will receive up to 12 one-month courses of continuous Optune treatment together with standard adjuvant temozolomide. The decision to add each additional treatment course will depend on the lack of treatment related serious adverse events which reappear upon re-challenge and lack of clinical disease progression. All technical aspects of the Optune device are handled by the DSS.

During Optune treatment the patient will be permitted to interrupt treatment for personal needs with the goal of achieving approximately 75% of the time over each 4-week period. Compliance reports with TTFields will be obtained by Novocure's DSS per usual clinical treatment procedures. If a patient's compliance is below 75% of the time over any 4-week period, the patient will be trained on the importance of compliance and any barriers to compliance will be addressed by the DSS. If a patient's compliance is below 50% of the time over any 4-week period, the patient will be trained on the importance of compliance and any barriers to compliance will be addressed by the DSS and study staff.

Approximately once per month, from baseline until treatment termination, all patients will report to an outpatient clinic where they will be assessed clinically and undergo routine laboratory examinations. The follow-up window for these visits is -3/+ 7 days for the Adjuvant TMZ follow-up window of approximately 12 months. The follow- up window after discontinuation of Adjuvant TMZ will be +/- 1 month. If patients are removed from study therapy due to toxicity and/or disease progression prior to the 2-year study treatment, the patients will have an end of study therapy visit within 1 month +/- 7 days. After this end of study therapy visit, patients will be followed by telephone interview every 3 months until death.

6.1.2.6.1 RECOMMENDATIONS FOR USE OF OPTUNE

All patients treated with Optune will be required to shave their heads to initiate array placement and Optune therapy. Array placement will be performed based on the transducer array map calculated during treatment planning. It is recommended that treatment with the device be continuous with breaks allowed for personal needs (e.g., showering, array exchange). Breaks should be no more than 1 hour twice daily.

Use compliance is aimed at a minimum of 50% (12 hours) with the goal of achieving at least 75% (18 hours) of the time per day on average. In addition, investigators may grant brief breaks in device treatment that last no more than 3 days in any 30-day period at the investigator's discretion. Subjects should be counseled by study staff if compliance reports show less than 50% compliance.

Optune is programmed by Novocure to deliver 200 kHz TTFields in two sequential, perpendicular field directions at a maximal intensity of 707 mARMS. There will be no adjustments made to the device by investigators or patients/caregivers.

It is recommended that patients replace the transducer arrays 2-3 times per week with the help of a caregiver. At each array replacement, it is recommended that the patient's scalp be re-shaved and skin treated according to the guidelines in section "Skin Care Guidelines."

There will be no dose adjustments to the device for adverse events. Reasons for breaks in treatment for longer than 24 hours will be documented.

Optune therapy will be stopped in the following cases:

- The occurrence of serious adverse events probably or definitely related to treatment with TTFields
- Clinical and functional deterioration considered by the investigator to be prohibitive of continuing treatment.
- After 24 months or after second progression, whichever occurs first.

Treatment with the Optune does not need to be terminated in the following situations:

- Toxicity due to temozolomide treatment
- Radiological progression alone will not lead to termination of TTFields treatment, but to replacement of the temozolomide treatment with best standard of care second line therapy:
    - Re-operation
    - Local radiation therapy
    - Second line therapy

6.1.2.6.2 SKIN CARE GUIDELINES

If the skin beneath the transducer arrays is inflamed, it is recommended that a prescription strength steroid ointment (e.g. 3% hydrocortisone or 0.05-0.1% Clobatasol) be prescribed to the patient. The patient or caregiver should apply the ointment after removing the arrays and cleaning the scalp with baby oil and gentle soap. The ointment should be left on the scalp for at least 30 minutes prior to washing the skin with a mild shampoo and applying a new set of arrays.

At each array replacement, it is recommended that the new set of arrays be shifted by approximately 2 cm compared to the previous layout so that the array discs are placed between the areas of skin irritation. At the next array replacement, the arrays should be shifted back to their original location.

If the dermis is breached (ulcers, open sores, punctate lesions, cuts, etc.), it is recommended that an antibiotic ointment (e.g. bactroban) be prescribed and used in place of the steroid ointment.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

Temozolomide is a standard of care therapy widely available throughout the U.S. Each site will utilize their hospital/local pharmacies for this study.

Optune devices will be delivered to patients and maintained by the Novocure network of Device Site Specialists across the U.S. Subjects will be met at their house and trained on device use. Device supplies will be delivered directly to the subject. The DSS will revisit as required for usage/compliance reporting, device maintenance, resupply, and to answer any questions the patient has about the device.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The complete package insert for temozolomide will accompany this protocol in lieu of an Investigator's Brochure.

The complete Optune Instructions for Use and Device Manual will accompany this protocol in lieu of a Device Manual.

Additionally, the Optune DSS may provide the patient with Optune Patient Information and Operation Manual, Optune Travel Support Brochure, Optune Patient Scalp Care Guidelines, etc. These are educational resources published by Novocure to better assist patients in understanding and using the device.

6.2.3 Product Storage and Stability

6.2.3.1 Temozolomide

Storage

- Store (temozolomide) Capsules at 25°C (77°F); excursions permitted to 15-30°C (59-86°F)
- Store (temozolomide) for Injection refrigerated at 2-8°C (36-46°F).
    - After reconstitution, store reconstituted product at room temperature (25°C [77°F]). Reconstituted product must be used within 14 hours, including infusion time.

The above storage guidelines are taken from the package insert; please see full insert for full details.

6.2.3.2 Optune

6.2.3.2.1 STORAGE CONDITIONS

- Temperature range: 23°F to 104°F for the device and additional parts
- Temperature range: 41°F to 81°F for the transducer arrays

- Relative humidity range: 15-75% for the device and additional parts
- Relative humidity range: 35-50% for the transducer arrays

6.2.3.2.2 TRANSPORT CONDITIONS

Transportation of the device and additional parts is possible using air/ground transportation in weather protected conditions as specified below:

- Temperature range: -13°F to 104°F
- Maximal relative humidity 15-75%
- No direct exposure to water

Transportation of the transducer arrays is possible using air/ground transportation in weather protected conditions as specified below:

- Temperature range: 32°F to 104°F
- Maximal relative humidity 15-75%
- No direct exposure to water

Please see the Optune Instructions for Use and Device Manual for full details.

6.2.4 Preparation

No preparation of either temozolomide or the Optune device is required by study staff. Temozolomide is administered per package insert. Optune is delivered, set-up, trained, and maintained by the Novocure DSS in the site's local area per clinical standard of care.

6.3 Correlative Studies

6.3.1 Tumor Tissue Specimens

6.3.1.1 Collection of specimens

Formalin fixed and paraffin embedded (FFPE) samples will be obtained from pathology or research tissue banks from primary tumors and first recurrent tumors, when available. One FFPE tissue block or fifteen slides of 20 µm thick section will be required for analysis. At least 20 pairs of primary tumors and first recurrent tumors from patients treated only with RT and concurrent temozolomide followed by adjuvant temozolomide, and not with TTFields or any other modalities (drug, radiation or device), are needed.

The Coordinating Center will obtain at least 20 historical paired samples from patients with the same tumor type or GBM before and after being treated with temozolomide and RT, but not with TTFields, over the last 5 years at the same participating institutions.

Sequencing analyses will be obtained by standard of care sequencing (immunohistochemistry or next-generation sequencing), from which mutation burden data will be obtained. Mutation burden will be expressed as number of mutations per Mb and will be extracted from sequencing reports for primary tumors and first recurrent tumors, where available.

6.3.1.2 Handling of specimens

FFPE samples, or pathology slides, will be used for standard H&E and immunohistochemical (IHC) staining for biomarkers for TILs and immunogenic cell death to be performed at the University of Florida.

IHC will be performed for the following, but not limited to, biomarkers: CD4, CD8, CD19, and CD45 (immune cells), AIM2, ACS and cGAS (immunogenic cell death), ISG15, INFalpha, and INFbeta (type 1 interferon response). Results will be expressed as percentage of cells with positive staining for each marker.

6.3.1.3 Shipping of specimens

FFPE or slide samples will be transported or mailed at ambient temperature to the University of Florida at the address below:

FORWARD Trial
c/o David Tran
1149 S Newell Drive, L3-132
Gainesville, FL 32610

Contact persons for tissue shipping and delivery:

Annie Allen:	Anne.Allen@neurosurgery.ufl.edu	phone #: 352-294-8836
Sonisha Warren:	Sonisha.Warren@neurosurgery.ufl.edu	phone #: 352-273-9000
Alexandra Sherman:	Alexandra.sherman@neurosurgery.ufl.edu	phone #: 352-273-6997
Nagheme Thomas:	Nagheme.Thomas@neurosurgery.ufl.edu	phone #: 352-273-6997

Shipments details should be logged into REDCap as soon as possible, and no later than 14 days from date of shipment.

6.3.2 Electric Field Modeling

Tumor Treating Fields are alternating electric fields at 200 kHz that can penetrate from the surface of the scalp into the brain to exert their anti-mitosis effect on dividing tumor cells. They have to first pass through the calvarium and subarachnoid space where they are attenuated by resistive property of bone and the conductive property of the cerebrospinal fluid. The remaining fields then reach the gray and white matters where they are either augmented or attenuated, depending on the local tissue properties and the geometry of structures such as the frontal, temporal and posterior horns of the lateral ventricles. As a result of these interferences, the amount of electric fields that reaches the gross tumor volume cannot be calculated precisely but can only be approximated numerically by methods such as finite element analysis [62].

6.3.2.1 Secondary imaging analyses

In addition, secondary analyses will be performed to determine the extent of correlation with respect to patient age, Karnofsky Performance Status, dexamethasone dosage, other non-therapeutic supportive medications and the number of cycles of chemotherapy, targeted therapy, biological agents or immune therapies received. Additional analyses will be conducted to also determine the correlation with fluctuations in laboratory values in complete blood counts, differential, electrolytes, liver function tests, as well as histological and genetic

parameters, such as Ki67, presence or absence of IDH-1 mutation, MGMT methylation status and/or somatic mutations found in next generation sequencing.

6.3.2.2 MRI Acquisition and DICOM images

The process of numerical estimation of electric field strength at the gross tumor volume consists of a number of steps. First, gadolinium-enhanced MP RAGE sequences will be acquired using MRI in one axis and reconstructed in the other two axes resulting in axial, coronal and sagittal images. Standard T2, FLAIR and pre-gadolinium sequences will also be obtained per local care standards. The field of view of the images should include the surface of the scalp (Fig. 7). These images will be exported as DICOM images.

Second, the DICOM images will be imported into a tissue segmentation software program as previously described [69]. Third, both intracranial and extracranial structures, including scalp, skull, dura, cerebrospinal fluid, gray matter, white matter and bilateral ventricles, will be segmented and meshed before solving using finite element methods [62].

Lastly, the electric field strength and the specific absorption ratio at the gross tumor volume will be tabulated using various electric field strength parameters, including EAUC, EV150, E95% and E50%, and specific absorption ratio parameters, including SARAUC, VSAR7.5, SAR95% and SAR50%. Together, these parameters will allow the comparison among patients of the electric field strength, as well as the amount of energy deposited, at the tumor.

The electric field and specific absorption ratio parameters will be correlated with measures of clinical outcome, including response rate, progression free survival and overall survival. Spearman rank-order correlation will be performed between the non-parametric electrical and clinical outcome variables.

The DICOM images will be obtained for analysis at baseline, each two months per protocol, and at the time of first and second progression. These analyses will be performed at Beth Israel Deaconess Medical Center. Analysis will be completed within two years of the last patient's enrollment.

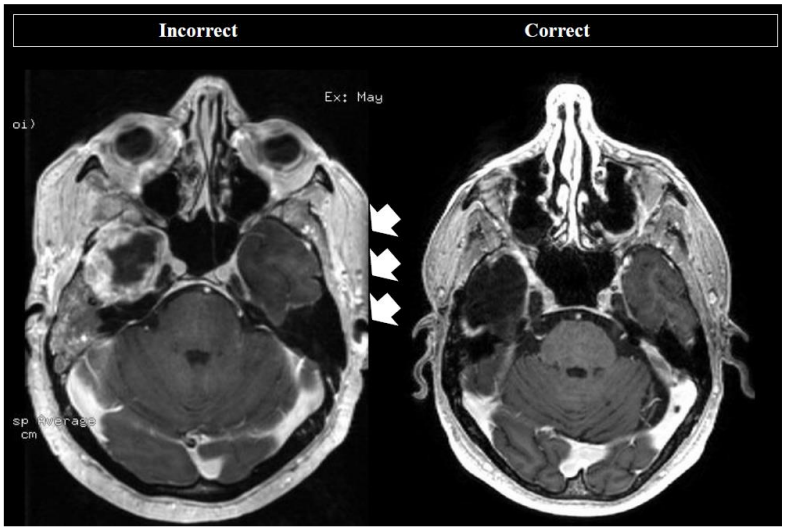

Figure 7. MRI image samples demonstrating field of view of the images must include the surface of the scalp. Incorrect image on the left does not include full scalp surface where indicated by arrows. The correct image at the right does include the surface of the scalp around the entire head.

6.3.2.3 Shipping of DICOM images

Anonymized DICOM images will be burned onto a compact disc coded with the following study information: subject's study number, Date of Service, and Site Number.

The following shipment time points will be used for each patient:

1. Baseline
2. First Progression
3. Second Progression

Images collected between shipping time points should be sent at the next shipment time point. Shipment information should be entered into the eCRF.

The CDs should be mailed to:

Brain Tumor Center
c/o Eric T Wong
Beth Israel Deaconess Medical Center
330 Brookline Avenue
Boston, MA 02215
Tel: 617-667-1665; Fax: 617-667-1664

Contact person:
Dr. Eric Wong: ewong@bidmc.harvard.edu

6.4 Study Intervention Compliance

Temozolomide compliance will be measured by subject self-report.

Optune compliance will be measured on each device and a monthly report provided to the subject and Site Investigator by the DSS.

Optune compliance will be addressed according to the following table:

TABLE 9: OPTUNE COMPLIANCE REQUIREMENTS

Subject Compliance in previous 4-week period per device report	DSS	Study Staff
≥ 75%	n/a	Reinforce good compliance report
74% to 50%	○ Re-train patient on device compliance requirements ○ barriers to compliance will be addressed	n/a
≤ 49%	○ Re-train patient on device compliance requirements ○ barriers to compliance will be addressed	Counsel subject during study visit re: need for additional use of device and identify any remaining barriers

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements.

Medication History should include the Chemoradiation treatment window, or at least 90 days of Medication History, whichever is greater.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Discontinuation from study treatment does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed.

Any serious adverse event deemed life threatening by the treating physician that is definitely related to the study device will be cause for immediate cessation of treatment for the patient. Patient follow up will continue after such an event.

7.2 Procedures for Orderly Termination of Participation

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- The investigator may remove a patient from the study in case of not complying with study protocol.
- If any clinical adverse event (AE), including a laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant

The reason for participant discontinuation or withdrawal from the study will be recorded on the electronic Case Report Form (eCRF). Subjects who sign the informed consent form and do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

7.3 Lost to Follow-Up

A participant will be considered lost to follow-up (LTFU) if he or she fails to return and is unable to be contacted by the study site staff for up to 180 days following his/her final visit or call.

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

- The site will attempt to contact the participant and reschedule the missed visit within the visit window, and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible this will include 3 telephone call attempts and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

Since the participant may be incapacitated, the LAR will be contacted in the event the participant cannot be reached.

Should the participant continue to be unreachable with no known outcome, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Efficacy Assessments

8.1.1 Day 0 – Start of TMZ Treatment Cycle 1

- Patients will begin treatment with temozolomide at 4 weeks (+14 days) from end of radiotherapy.
- Cycle 1 will be dosed at 150mg/m^2 daily for 5 consecutive days of a 28-day cycle.
- Optune therapy should start within two weeks of start of adjuvant temozolomide, but not more than 7 weeks from the end of temozolomide/RT and will be continuous throughout study.
- Review Concomitant Medications
- Update Adverse Events

8.1.2 Week 4 (-3/+7 days) - Start of Subsequent TMZ Treatment Cycles, repeat every 4 weeks up to 12 cycles

- Physical Exam
- Patients will begin treatment with temozolomide cycle 2 and subsequent cycles every 4 weeks (-3/+7 days) at 200 mg/m^2, if the CTC non-hematologic toxicity for Cycle 1 is Grade ≤2 (except for alopecia, nausea and vomiting), absolute neutrophil count (ANC) is ≥ 1.5 x 10^9/L, and the platelet count is ≥ 100 x 10^9/L.
    - The dose remains at 200 mg/m^2 per day for the first 5 days of each subsequent cycle unless toxicity occurs. If the dose was not escalated at Cycle 2, escalation should not be done in subsequent cycles.
- Neurological status and KPS
- CBC with differential
- Biochemistry Panel (electrolytes, creatinine, bilirubin, liver enzymes)
- Steroid Dose (recorded)
- Optune therapy will be continuous throughout study. Compliance documentation will be obtained every 4 weeks (-3/+ 7 days).
- Review Concomitant Medications
- Update Adverse Events

8.1.3 Week 8 (+/- 1 week) – repeat MRIs every 8 weeks only, until treatment stop

- Contrast-enhanced MRI of the brain
- Quality of life questionnaires (EORTC QLQ-C30/BN20, and MMSE)
- RANO assessment
- Review Concomitant Medications
- Update Adverse Events

8.1.4 Progression

If progression is identified, the following procedures should be performed:

- Contrast-enhanced MRI of the brain
- Physical Exam
- Neurological status and KPS
- CBC with differential
- Steroid Dose (recorded)
- Biochemistry panel (Electrolytes, creatinine, bilirubin, liver enzymes)
- Quality of life questionnaires (EORTC QLQ-C30/BN20, and MMSE)
- RANO assessment
- Optune Therapy – confirm continuation (See Recommendations for Use of Optune)
- Optune Compliance
- Review Concomitant Medications
- Update Adverse Events
- Tumor Tissue sample – determining availability of FFPE, pathology slides and/or frozen sample(s) for first recurrence

8.2 Safety and Other Assessments

The following labs are collected for safety by the investigator or his/her designee:

- CBC with differential
- Pregnancy Test (urine or serum) – WOCBP only
- Biochemistry (electrolytes, creatinine, bilirubin, liver enzymes)

Lab results should be reviewed promptly.

8.2.1 After Treatment Stop

Week 4 (+/- 7 days) from treatment stop

The following will be performed 4 weeks (+/- 7 days) from treatment stop:

- Physical exam
- Neurological status and KPS
- CBC with differential
- Biochemistry Panel (electrolytes, creatinine, bilirubin, liver enzymes)
- Review Concomitant Medications
- Update Adverse Events

8.2.2 Survival Follow-up

Every 3 months +/- 14 days

After the final visit is conducted, subjects will be followed every 90 days (3 months) +/- 14 days, either by phone or in-person.

See the Survival Follow-up Telephone Script for specific questions.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3 Classification of an Adverse Event

8.3.3.1 Severity of Event

AEs will be assessed according to the CTCAE version 5.0 (term and grade) and documented. If CTCAE term does not exist, the PI should select the most appropriate CTC category and indicate “other”.

If CTCAE grading does not exist for an AE, the severity of the AE will be graded as:

1. Mild - Events require minimal or no treatment and do not interfere with the participant’s daily activities.

2. Moderate - Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

3. Severe – Events interrupt a participant’s usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term “severe” does not necessarily equate to “serious”

4. Life-threatening

5. Fatal

8.3.3.1.1 CEREBRAL EDEMA TOXICITY EXCEPTION:

NCI CTCAE criteria categorize all cerebral edema as grades 3-5. Cerebral edema, with or without mass effect, normally presents in glioma patients as part of the disease process and can be exacerbated by standard of care chemotherapy and radiation. Therefore, cerebral edema, for purposes of this protocol, although ranked grades 3-5 by NCI CTCAE criteria, will be graded with the full range of toxicities in order to accurately capture the AE: (1) Mild (2) Moderate (3) Severe (4) Life Threatening (5) Fatal.

8.3.3.1.2 CLINICAL SIGNIFICANCE OF LAB RESULTS

Only clinically significant lab abnormalities will be recorded as AEs.

Abnormal laboratory results shall be assessed by the Site investigator (or designee) promptly for clinical significance. Laboratory reports should be initialed and dated by the evaluator.

An abnormal lab value should be deemed clinically significant if both of the following conditions are met:

- The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline.
- The abnormality is of a degree that requires additional active management, e.g., change of dose, discontinuation of the drug, close observation, more frequent follow-up assessments, or further diagnostic investigation.

8.3.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the site investigator (clinician) who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study intervention must always be suspect.

- Definitely Related (Definite) – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- Probably Related (Probable) – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- Potentially Related (Possible) – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other

drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- Not Related (Unrelated) – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.3.3.3 Expectedness

Site Investigators will be responsible for determining whether an adverse event (AE) is expected or unexpected.

An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 Summary of AE Reporting Requirements for Site Investigators

8.3.4.1 Adverse Event Reporting

All Adverse Events CTCAE Grades 1 and 2 should be entered into the eCRF within 14 days of discovery by the site if possibly, probably, or definitely related to the study treatment in the opinion of the site investigator.

All Adverse Events CTCAE Grade 3 and higher should be entered into the eCRF within 14 days of discovery by the site.

8.3.4.2 Serious Adverse Event Reporting

All SAEs should be reported to the Coordinating Center within 24 hours of discovery by the site.

Per UF DSMB policy, all SAEs will be reported to the UFHCC Data Integrity and Safety Committee (DISC) within 5 days.

8.3.5 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Study Staff will record all reportable events with start dates occurring any time after the start of study treatment for 30 days after the After Treatment Stop Visit. At each study visit, the investigator or his/her designee will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

During Survival Follow-up, AEs and SAEs will not be collected. Only Progression status and survival outcomes will be monitored during this stage of the study.

8.3.6 Adverse Event Reporting

Adverse Events shall be recorded and entered into the eCRF within 14 days. The Coordinating Center will report the events to the DSMB for review per policy.

The site investigator is responsible for informing the local IRB of adverse events per local requirements.

8.3.7 Serious Adverse Event Reporting

The site investigator must report any serious adverse event, whether or not considered study intervention related, including those listed in the protocol, package insert, or device use manual to the Coordinating Center within 24 hours of discovery and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the Coordinating Center.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Supporting documentation of the event may be requested by the Coordinating Center and should be provided as requested.

8.3.7.1 Unanticipated Adverse Device Effect

The investigational device exemption (IDE) regulations define an unanticipated adverse device effect (UADE) as “any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects” (21 CFR 812.3(s)). UADEs must be reported by the clinical investigator to the Coordinating Center and the reviewing IRB, as described below:

Investigators are required to submit a report of a UADE to the Coordinating Center within 24 hours after the investigator first learns of the event.

The IDE Sponsor will conduct an evaluation of a UADE and will report the results of the evaluation to FDA, all reviewing IRBs, and participating investigators within 10 working days after the sponsor first receives notice of the effect.

The site investigator is responsible for informing the IRB of the UADE per local requirements.

The IDE sponsor will make reports to FDA as required by §812.150.

8.3.8 Reporting Events to Participants

If additional risks are identified, the protocol will be amended and Informed Consent Form revision(s) implemented.

8.3.9 Events of Special Interest

This section is not-applicable. There are no events of special interest for this study. All adverse events CTCAE Grade 3 and above, and serious adverse events shall be collected and reported as describe in this protocol.

8.3.10 Reporting of Pregnancy

Pregnancy of any female subject including pregnancies that occur in the female partner of a male study subject should be reported to the Coordinating Center within 24 hours of discovery. All pregnancies must be followed to outcome.

The subject will be withdrawn from the study and the site investigator will continue safety follow-up.

8.4 Unanticipated Problems

8.4.1 Definition of Unanticipated Problems (UP)

Unanticipated problems involving risks to participants or others includes, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

This definition could include an unanticipated adverse device effect, any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (21 CFR 812.3(s)).

8.4.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) per local policy and to the Coordinating Center within 24 hours of discovery. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;

- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

An investigator shall submit to the reviewing Institutional Review Board (IRB) a report of any unanticipated adverse device effect occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect (21 CFR 812.150(a)(1) and to the Coordinating Center within 24 hours of discovery.

The IDE sponsor who conducts an evaluation of an unanticipated adverse device effect under 812.46(b) will report the results of such evaluation to the Food and Drug Administration (FDA) and to all reviewing IRB's and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter the IDE sponsor shall submit such additional reports concerning the effect as FDA requests (21 CFR 812.150(b)(1)).

8.4.3 Reporting Unanticipated Problems to Participants

If additional risks are identified, the protocol will be amended and Informed Consent Form revision(s) implemented.

9 CONTROL COHORT

This trial will include a control cohort that includes both historical and contemporaneous control patients. A minimum of 90 and a maximum of 100 patients will be enrolled in the control cohort. This is a non-interventional control group (data collection only). All patients in the control cohort will be enrolled at the same participating institutions. Risks associated with participation of the control cohort are minimal.

Historical controls will be gathered via chart review of patients completing chemo/RT after 1/1/2016 and before 5/20/2019; 5/20/2019 is the initial date of IRB approval for the coordinating center and first opened study site. Control patients completing chemo/RT after 5/20/2019 will be enrolled as contemporaneous controls.

Control subjects (contemporaneous or historical) will be enrolled via informed consent if consent is practicable. An IRB waiver of consent will be requested for historical control patients who may be deceased, no longer being followed or lost to follow-up.

9.1 Control Cohort Rationale

Identification of a homogeneous historical cohort for comparing the investigational device data to historical data is of critical importance to this study's design and scientific rigor. This historical and contemporaneous cohort will include patients completing radiation and chemotherapy treatment no earlier than January 1, 2016, when chemotherapy became the care standard in these tumor subtypes [16].

9.2 Control Cohort Inclusion Criteria

In order to be eligible to enroll in the control cohort, an individual must meet all of the following criteria:

A. Willing and able to provide written informed consent or eligible for inclusion under an IRB-approved waiver of consent
B. Male or female, 18 years or older

C. Histologic confirmation of WHO Grade II or III astrocytoma
    a. mixed oligoastrocytomas are permitted
D. Mutational identity determined by CLIA-certified sequencing including:
    a. IDH1/2 wildtype (i.e. lack of detectable mutations on the sequencing report, variants of unknown significance or non-synonymous single nucleotide polymorphisms deviating from wild-type sequence do not apply)

    AND

    b. TERT promoter mutation(s)
E. Karnofsky performance status ≥70%
F. Maximal safe resection
    a. biopsy alone is allowed
G. Completed standard chemoradiation and concurrent temozolomide and adjuvant temozolomide.
    a. Patients with a tumor that was biopsied or resected in the past followed by observation only without definitive chemoradiation and/or chemotherapy given will be eligible, as long as: repeat maximal surgical resection (biopsy only allowed) has been performed, definitive temozolomide/RT treatment meets the criteria above, and adjuvant temozolomide treatment was given (or planned for the contemporaneous cohort)
H. Candidate for adjuvant high dose temozolomide per investigator's clinical judgement (for the contemporaneous cohort)
I. No evidence of early disease progression per RANO criteria upon completion of Chemo/RT and beginning adjuvant temozolomide

9.3 Control Cohort Exclusion Criteria

At time of starting adjuvant (first-line) therapy:

L. Prior treatment with anti-angiogenic agents including bevacizumab.
M. Progressive disease (according to RANO criteria) after temozolomide/RT, at time of starting adjuvant therapy.
N. Actively participating in another clinical treatment trial intended to treat the underlying astrocytoma at start of adjuvant Temozolomide treatment.

9.4 Control Cohort Lifestyle Considerations

- Patients enrolled in the control cohort must not have any past, present, or planned TT Fields treatment.

9.5 Control Cohort Limited Data Collection

Data collected for the historical and contemporaneous control group will be distinct from data collected for interventional patients in this study, as described below. Data for control patients is designed to be collected exclusively from chart review.

All control cohort documentation should be uploaded into the eCRF within 45 days of collection. Data extracted from the medical record shall be defined as collected on the day of extraction.

1. Informed Consent or waiver
2. Demographics

3. Pathology
4. Date of and extent of operation (GTR, partial, or biopsy)
5. Summary of Concurrent Chemo/RT
6. KPS
7. Summary of Adjuvant Chemotherapy (TMZ dates, number of cycles)
8. Summary of Additional Therapies (therapy types, dates, number of cycles)
9. Date(s) of Progression
10. Date of Death (if applicable)
11. Tumor tissue samples (optional)

Tumor Tissue samples are optional for control cohort patients. Adverse Events are not collected, and not reportable for control patients. MRI Correlative Study does not include control cohort participants. Concomitant Medications will not be maintained for control patients, although applicable steroid doses may be recorded as part of the RANO form.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical Hypotheses

- Primary Efficacy Endpoint: overall survival (OS)

Control arm: the control arm will consist of both historical and contemporaneous control patients. Historical controls will consist of TERT mutation-only astrocytoma patients treated and followed by the participating sites between January 1, 2016 and May 20, 2019. As described above, these patients will have received standard treatment and would have met first tier inclusion/exclusion criteria for the study. Data for these patients will be collected via chart review. Contemporaneous controls will also consist of patients who qualify for the trial and receive standard treatment but decide not to proceed with the Optune experimental treatment because of personal preference. This personal preference must be non-medical in nature. Based on clinical experience, most commonly this non-medical patient personal preference preventing Optune use will be unwillingness to shave his/her head and/or unwillingness to wear a visible medical device on his/her head most of the day.

- N = 90 patients total in an expected mixture ranging between 80 historical controls and 10 contemporaneous controls to 60 historical controls and 30 contemporaneous controls.

- Assumed median survival = 22 months (based on Figure 3A in Eckel-Passow et al [12])

- Follow-up = up to 60 months for the historical controls, up to 60 months for the contemporaneous controls

Experimental treatment arm: TERT mutation-only astrocytoma patients who will receive standard treatment plus TTFields therapy at one of 15 participating centers.

- N = 90 patients

- Assumed accrual rate = 2.5 patients/month accumulated over 15 centers
- Accrual period = 36 months
- Follow-up period after the end of accrual = 24 months
- Assumed survival time distribution = Weibull with accelerating hazard and proportional hazards

10.2 Sample Size Determination

To assess sample size requirements for detecting an improvement in median overall survival (OS) in the experimental treatment arm relative to the historical control arm, we initially assumed 60 to 90 patients receiving standard treatment and follow-up between 1-2016 and 5-2019 would be available for chart review. We assumed that these historical control patients would have a maximum follow-up time of 60 months. For the experimental treatment, we assumed an accrual rate of 2.5 patients per month across 15 participating centers and an accrual period of 36 months, yielding an enrollment of 90 patients in the experimental arm. The patients in the experimental arm would be followed for an additional 24 months after the end of accrual. We considered improvements in median survival ranging from 4 to 8 months to be of clinical interest. We also assumed that survival times would follow a Weibull distribution with proportional hazards and an accelerating hazard function (shape parameter k=2.0, estimated from Figure 3A in Eckel-Passow et al [12]. We also considered a more slowly accelerating Weibull shape parameter of k=1.8. Under these assumptions, the power to detect ***improvements*** in median OS using a one-sided log-rank test (one-sided alpha=0.05) are summarized in Table 10 for selected combinations of historical control sample size, Weibull shape parameter, and number of months improvement in median OS [detectable effect size methodology adapted from (Wu and Xiong, 2014)]. We then explored the effect of mixing historical and contemporaneous controls in select mixtures into a single control arm, noting a very slight improvement in power to detect improvements in survival. Overall, a control arm sample size of 90 patients, in historical/contemporaneous control mixtures of 90+0, 80+10, 70+20, and 60+30 all have at least 80% power to detect improvements in survival between 6 and 8 months.

TABLE 10: POWER TO DETECT M MONTHS IMPROVEMENT IN MEDIAN OVERALL SURVIVAL UNDER SELECTED SCENARIOS

Accrual rate (N/mo)	Accrual period (mos)	Follow-up period (mos)	Trial N	Hx Ctl+ Contemp Ctl N	Weibull shape k	Power to detect M months improvement in median OS at one-sided α=0.05 using the log-rank test				
						M = 4	5	6	7	8
2.5	36	24	90	50+ 0	2.0	0.525	0.667	0.782	0.866	0.922
2.5	36	24	90	60+ 0	2.0	0.565	0.711	0.823	0.899	0.946
2.5	36	24	90	70+ 0	2.0	0.598	0.745	0.853	0.922	0.962
2.5	36	24	90	80+ 0	2.0	0.626	0.773	0.876	0.938	0.972
2.5	36	24	90	90+ 0	2.0	0.649	0.796	0.894	0.950	0.979
2.8	36	24	90	80+10	2.0	0.650	0.796	0.894	0.950	0.979
3.1	36	24	90	70+20	2.0	0.651	0.797	0.895	0.951	0.979

3.3	36	24	90	60+30	2.0	0.652	0.798	0.895	0.951	0.979
2.5	36	24	90	50	1.8	0.447	0.577	0.692	0.787	0.858
2.5	36	24	90	60	1.8	0.483	0.620	0.737	0.828	0.892
2.5	36	24	90	70	1.8	0.513	0.654	0.771	0.858	0.916
2.5	36	24	90	80	1.8	0.538	0.683	0.798	0.880	0.933
2.5	36	24	90	90	1.8	0.560	0.707	0.820	0.898	0.946
2.5	36	24	90	100	1.8	0.579	0.727	0.838	0.912	0.955
2.8	36	24	90	80+10	1.8	0.561	0.708	0.821	0.899	0.946
3.1	36	24	90	70+20	1.8	0.562	0.709	0.822	0.899	0.947
3.3	36	24	90	60+30	1.8	0.563	0.710	0.823	0.900	0.947

10.2.1 Analysis of Primary and Secondary Endpoints and Correlative Studies

We will compare OS and progression-free survival (PFS) between historical control and experimental treatment arms using the log-rank test (we will also collect PFS data via chart review for the historical control patients). For our correlative studies, we will use the independent-sample t test to compare mean mutation burden between patient samples treated with RT+temozolomide or RT+temozolomide+TTFields. We may perform the same comparison within a linear regression framework if the need arises to adjust for any potential confounders for the effect of TTFields in our correlative studies.

To investigate the influence of electric field and energy deposition in the tumor on outcomes, we will use the Wilcoxon rank sum test to compare the electric field and specific absorption ratio parameters between: responders and non-responders; subjects with progression-free survival ≥ and < 1 year; subjects with overall survival ≥ and < 2 years; and subjects with overall survival ≥ and < 1.5 years. We will estimate Pearson correlation coefficients to assess the correlation of OS and PFS with electric field or specific absorption ratio parameters.

We may stratify the patients by MGMT methylation status, extent of resection, or a combination of the two.

10.3 Populations for Analyses

The historical control arm will consist of patients with tissue-based diagnosis of Grade II or III astrocytomas, 18 years or older, of both genders, after surgery or biopsy followed by radiation therapy with concurrent temozolomide. As described above, these patients will have received standard treatment and would have met first tier inclusion/exclusion criteria for the study. Data for these patients will be collected via chart review. Contemporaneous controls will also consist of patients who qualify for the trial and receive standard treatment but decide not to proceed with the Optune experimental treatment because of personal preference. This personal preference must be non-medical in nature. Based on clinical experience, most commonly this non-medical patient personal preference preventing Optune use will be unwillingness to shave his/her head and/or unwillingness to wear a visible medical device on his/her head most of the day.

The interventional patient population will consist of patients with tissue-based diagnosis of Grade II or III astrocytomas, 18 years or older, of both genders after surgery or biopsy followed by radiation therapy with concurrent temozolomide and subsequent TTFields therapy plus temozolomide.

10.4 Safety Analyses

Clinical and laboratory test parameters monitored for the purpose of identifying severe adverse events (SAEs) will be summarized at the end of the trial using standard summary statistics and confidence intervals. The cumulative incidence of SAEs will be monitored continuously within the framework of a stopping rule.

Stopping Rule: We will use separate sequential Pocock-type boundaries [82] to monitor continuously for chemotherapy-related and device-related SAEs. If the cumulative number of patients experiencing an SAE is equal to or exceeds the relevant boundary indexed by the number of patients currently enrolled, a halt in enrollment will be triggered for further evaluation, to determine if the study should be discontinued for safety reasons.

The maximum acceptable toxicity rate will be set at 25% for device-related SAEs, and 50% for chemotherapy-related SAEs.

Each type of SAE will have its own boundary, which will be specified so that the overall probability of stopping the trial early for each type of SAE is 0.05 when the true device-related rate is less than 25% or the true chemotherapy-related rate is less than 50%.

TABLE 11: POCOCK BOUNDARY STOPPING RULE FOR DEVICE-RELATED SAE MONITORING

(SAE target rate=25%, N=90 patients in the treatment arm)

Number of Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
SAE Boundary	-	-	-	4	5	5	6	6	7	7	7	8	8	9	9	9	10	10	10	11

Number of Patients	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
SAE Boundary	11	11	12	12	13	13	13	14	14	14	15	15	15	16	16	16	17	17	17	18

Number of Patients	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
SAE Boundary	18	18	19	19	19	20	20	20	21	21	21	22	22	22	23	23	23	23	24	24

Number of Patients	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
SAE Boundary	24	25	25	25	26	26	26	27	27	27	28	28	28	28	29	29	29	30	30	30

Number of Patients	81	82	83	84	85	86	87	88	89	90
SAE Boundary	31	31	31	32	32	32	32	33	33	33

(Stop trial if cumulative number of patients experiencing a device-related SAE is equal to or exceeds the SAE event boundary)

TABLE 12. POCOCK BOUNDARY STOPPING RULE FOR CHEMOTHERAPY-RELATED SAE MONITORING

(SAE target rate=50%, N=90 patients in the treatment arm; will be expanded to include contemporaneous controls if enrolled)

Number of Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
SAE Boundary	-	-	-	-	-	-	7	8	9	10	10	11	12	12	13	14	14	15	15	16

Number of Patients	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
SAE Boundary	17	17	18	19	19	20	20	21	22	22	23	23	24	25	25	26	27	27	28	28

Number of Patients	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
SAE Boundary	29	29	30	31	31	32	32	33	34	34	35	35	36	36	37	38	38	39	39	40

Number of Patients	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
SAE Boundary	41	41	42	42	43	43	44	45	45	46	46	47	47	48	49	49	50	50	51	51

Number of Patients	81	82	83	84	85	86	87	88	89	90
SAE Boundary	52	52	53	54	54	55	55	56	56	57

(Stop trial if cumulative number of patients experiencing a device-related SAE is equal to or exceeds the SAE event boundary)

11 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

11.1 Regulatory, Ethical, and Study Oversight Considerations

11.1.1 Informed Consent Process

11.1.1.1 Consent/assent and Other Informational Documents Provided to participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The following consent materials are submitted with this protocol:

- Informed Consent Form – Coordinating Center Template
- Informed Consent Form (Control Arm) – Coordinating Center Template

11.1.1.2 Consent Procedures and Documentation

No clinical investigator may involve a human being as a subject in research unless the investigator has obtained the legally effective informed consent from the subject, unless waived by the IRB. Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures.

11.1.2 Study Discontinuation and Closure

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

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping

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

11.1.3 Confidentiality and Privacy

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

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

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

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

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

11.1.4 Future Use of Stored Specimens and Data

11.1.4.1 MRI Data

MRI Data collected for this study will be analyzed and stored at Beth Israel Deaconess Medical Center. After the study is completed, the de-identified, archived data will be stored per local institution guidelines. Permission to transmit data to the Beth Israel Deaconess Medical Center will be included in the informed consent.

11.1.4.2 Tissue samples

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be shipped to the University of Florida for analysis. These samples could be used to research the causes of glioma, its complications and other conditions for which individuals with glioma are at increased risk, and to improve treatment. The researchers will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant during laboratory analyses.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens collected. However, withdrawal of consent to biosamples already collected will not be possible after analysis is underway.

When the study is completed, access to study data will be provided through the University of Florida. Residual samples will be returned or destroyed per standard practice.

11.1.5 Key Roles and Study Governance

Study Chair
David D. Tran, MD, PhD
University of Florida
PO Box 100265
Gainesville, FL 32610
(352) 273-9000
David.Tran@neurosurgery.ufl.edu

Coordinating Center PI
Ashley Ghiaseddin, M.D.
University of Florida
PO Box 100265
Gainesville, FL 32610
(352) 273-9000
Ashley.Ghiaseddin@neurosurgery.ufl.edu

11.1.6 Safety Oversight

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including oncology expertise. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of

interest. The DSMB will meet at least annually to assess safety and efficacy data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the Principal Investigator and IRB.

11.1.7 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with Good Clinical Practice (GCP), and with applicable regulatory requirement(s).

- Monitoring and auditing for this study will be performed by the UF Coordinating Center.
- Details of ongoing site monitoring are documented in a Clinical Monitoring Plan (CMP).
    - A blend of centralized and on-site monitoring may be utilized based on site enrollment.
    - Monitoring will focus on targeted validation of primary outcome measures and any key safety measures determined throughout the study by DSMB. Validation will take place in the REDCap database, which will serve as the eCRF and source document repository for the study.
- Independent audits will be conducted by the UF Health Cancer Center at least annually.
    - Details of the auditing plan are documented in the UF Health Cancer Center manual.

11.1.8 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan should be on file to describe a site's ongoing quality management activities.

Quality control (QC) procedures will be implemented by the Coordinating Center beginning with the data entry system and data QC checks. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution; these data items should be addressed within 14 days of site notification.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice (GCP), and applicable regulatory requirements.

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

11.1.9 Data Handling and Record Keeping

11.1.9.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived

from source documents should be consistent with the data recorded on the source documents. It is permissible to use site worksheets or EMR records in place of visit worksheets if the same information is documented.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into REDCap, a 21 CFR Part 11-compliant data capture system provided by the University of Florida Coordinating Center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

Data should be entered into REDCap, including uploading related source documents, within 14 days of data collection.

For urgent data transmissions (Serious Adverse Events, etc.), fax and/or secure email transmission is acceptable with separate notification to the Coordinating Center confirming receipt of the records for review.

Fax: (352) 392-8413
Separate notification via email: *Forward-study@ufl.edu* or via phone: (352) 294-8836.

11.1.9.2 Study Records Retention

Study documents should be retained for at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

Patients will be followed for survival status until death, loss to follow-up, withdrawal, or until study data collection is concluded. As stated earlier, it is anticipated that data collection will be complete in 5 years. At that time, the data set will then be cleaned and locked, and identifiable patient data will be coded for analysis.

11.1.10 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with GCP:

- Compliance with Protocol
- Quality Assurance and Quality Control
- Identification and correction of Noncompliance

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 14 days of identification of the protocol deviation, or within 14 days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, and reported to Coordinating Center via the eCRF. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

11.1.11 Publication and Data Sharing Policy

The results of the clinical investigation will be made publicly available in case of positive or negative results following the completion of the trial.

11.1.12 Conflict of Interest Policy

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

Dr. David Tran, the Study Chair and Sub-Investigator at the University of Florida, is a paid member of the medical advisory board of Novocure which provides funding for the study.

11.2 Additional Considerations

11.2.1 Guidance on Contraception

For the purposes of the proposed study, “highly effective” contraceptive methods are defined as those, alone or in combination, that result in a low failure rate (i.e., less than 1 percent per year) when used consistently and correctly, and include the following:

- Surgical sterilization at least 6 months before Study Drug administration
- Implants
- Levonorgestrel (LNG) and Copper T IUDs
- Sexual abstinence

Subjects who prefer methods which evidence a higher (6-9%) failure rate with typical use will be required to employ at least two methods of contraception concurrently. These methods include the following:

- Injectable hormone depos
- Oral contraceptive pill
- Hormone patch
- Vaginal ring

Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are NOT acceptable methods of contraception.

http://www.cdc.gov/reproductivehealth/unintendedpregnancy/pdf/contraceptive_methods_508.pdf for a list of contraceptive methods and effectiveness.

11.2.2 Enrollment Documentation Requirements

The following documentation is required for subject enrollment. All documents should be uploaded into the eCRF within 14 days of enrollment.

1. Signed and dated Informed Consent Form
2. Documentation of consent
3. Pathology Report including MGMT methylation status. If not feasible or indeterminate, must be stated. Indeterminate methylation status will be considered unmethylated.
4. Immunohistochemistry or next-generation sequencing Report (e.g. Foundation Medicine, TEMPUS, Caris, or similar CLIA-certified sequencing service)
5. Operative Report of biopsy or resection
6. Chemo/Radiation Radiology Report confirming total Gy delivered
    a. An isodose map of the treatment area may be required (to adjudicate cases with suspected/possible new lesions outside the RT treatment field) before eligibility can be confirmed
7. Oncology Screening Visit Note
8. Screening MRI Report; RANO forms are not required at time of enrollment.
9. Screening labs with local lab reference ranges, reviewed and signed by local investigator
10. Relevant medical history
11. Concurrent medications
12. Steroid dose
13. Signed and dated enrollment checklist

11.2.2.1 Control Cohort Enrollment Documentation Requirements

The following documentation is required for control subject enrollment. All documents should be uploaded into the eCRF within 45 days of enrollment.

1. Signed and dated Informed Consent Form
2. Documentation of consent checklist
3. Pathology Report including MGMT methylation status. If not feasible or indeterminate, must be stated. Indeterminate methylation status will be considered unmethylated.
4. Immunohistochemistry or next-generation sequencing Report (e.g. Foundation Medicine, TEMPUS, Caris, or similar CLIA-certified sequencing service)
5. Screening MRI Report; RANO forms are not required at time of enrollment.
6. Signed and dated enrollment checklist

    Additionally, the following data shall be entered into the eCRF at enrollment and source documentation included:
    a. KPS
    b. Resection Summary
    c. Chemo/Radiation Summary

11.3 Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
CBC	Complete blood count
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CRF	Case report form
CT	Computed tomography
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
DICOM	Digital Imaging and Communications in Medicine
DISC	Data Integrity and Safety Committee
DOB	Date of birth
DSMB	Data and Safety Monitoring Board
DSS	Device Support Specialist
DTIC	Dacarbazine
eCRF	Electronic case report form
EGFR	Estimated Glomerular Filtration Rate
EORTC QLQ-C30	European Organization for Research and Treatment of Cancer Quality of Life Questionnaire - Cancer
EORTC QLQ-BN20	European Organization for Research and Treatment of Cancer Quality of Life Questionnaire - Brain
FDA	Food and Drug Administration
FFPE	Formalin fixed and paraffin embedded
FISH	Fluorescence In Situ Hybridization
FLAIR	Fluid Attenuated Inversion Recovery
FSH	Follicle-stimulating hormone
GBM	Glioblastoma multiforme
HRT	Hormone replacement therapy
ICIs	Immune checkpoint inhibitors
IDH	Isocitrate dehydrogenase
IHC	Immunohistochemical
IRB	Institutional Review Board
kHz	kilohertz
KPS	Karnofsky performance status
LAR	Legally Authorized Representative
LTFU	Lost to Follow-up
MGMT	O6-alkylguanine DNA alkyltransferase
MHz	Megahertz
MMSE	Mini-Mental State Examination
MP RAGE	Magnetisation-prepared rapid gradient-echo
MRI	Magnetic resonance imaging
NHEJ	Non-Homologous End Joining
OHRP	Office of Human Research Protections
OS	Overall survival
PCV	Procarbazine/CCNU/vincristine

PFS	Progression-free survival
PT	Prothrombin time
PTT	Partial thromboplastic time
QoL	Quality of Life
RANO	Response Assessment in Neuro-Oncology
RECIST	Response Evaluation Criteria In Solid Tumors
RR	Radiological Response
RT	Radiation Therapy
SAE	Serious adverse event
WOCBP	Women Of Child-Bearing Potential

11.3.1 Summary of Figures

11.4 Protocol Amendment History

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Section	Description of Change	Brief Rationale
1.1	5 April 2019	Title Page	Addition of NCT and IDE numbers	Updates flowing from IDE approval
1.1	5 April 2019	1.1	Clarification/Correction of enrollment vs. participant timelines	Response to FDA initial review question
1.1	5 April 2019	2.1.1.1	Addition of generalizability of results section	Clarification of potential benefits to the Medicare population from study results
1.1	5 April 2019	10.1.11	Revision of Publication Policy	Updated to reflect the current study contract
1.1	5 April 2019	1.3, 5.5.1, 6.3.1, 8.1.4	Consistent description of acceptable tissue sample types	FFPE, pathology slides, or frozen samples are acceptable for onsite tissue verification; only FFPE and pathology slides may be shipped
2.1	16 Dec 2019	1.1, 9, 10, 10.3, 11.1.1	Addition of Control Cohort	Methodological improvement in the historical control for this study
2.1	16 Dec 2019	4.1.3, 5.1, 5.5	Removal of Inclusion criteria regarding 1p/19q and/or ATRX	Update of WHO Classification of Grade II and III criteria for gliomas
2.1	16 Dec 2019	5.1	Addition of Inclusion criteria	Clarify patients appropriate for Optune
2.1	16 Dec 2019	1.1, 10.3	Removal of Stupp Protocol specification	Avoid any specific Gy requirement assumption analogous with Stupp
2.1	16 Dec 2019	6.1.1.3	Addition of RANO clarification regarding non-enhancing disease	Clarification of criteria for non-enhancing disease via FLAIR
2.1	16 Dec 2019	10.2.1	Addition of stratification by extent of resection	Implementation of FDA suggestion
2.1	16 Dec 2019	1.3, 5.1, 5.4, 6.1, 6.2, 6.3, 8.1.2	Minor Typographical Updates	Clarification
2.1	16 Dec 2019	11.1.6, 11.1.7	Update DSMB audit frequency	Updated to reflect current study approved risk level (moderate) and related annual DSMB audit frequency
2.1	16 Dec 2019	Sections not applicable to this protocol (and therefore intentionally left blank)	Removed to avoid confusion	Implementation of SRMC suggestion for readability.

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