Phase 2, Single Arm, Historically Controlled Study Testing The Safety and Efficacy of Adjuvant Temozolomide Plus TTFields (Optune®) Plus Pembrolizumab in Patients with Newly Diagnosed Glioblastoma (2-THE-TOP)

THERAPEUTIC PROTOCOL

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Sponsor: Novocure, Inc.

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Protocol Revision History

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8/28/2017	Initial Protocol Release (SRMC Approval Only)
11/06/2017	Amendment #1 Version 2.0 (FDA determination)
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07/17/2020	Amendment #10 Version 9.2
3/17/2021	Amendment #11 Version 9.3
12/16/2021	Amendment #12 Version 9.4
1/28/2022	Amendment #13 Version 9.5
9/28/2022	Amendment #14 Version 10.0
6/09/2023	Amendment #15 Version 10.1

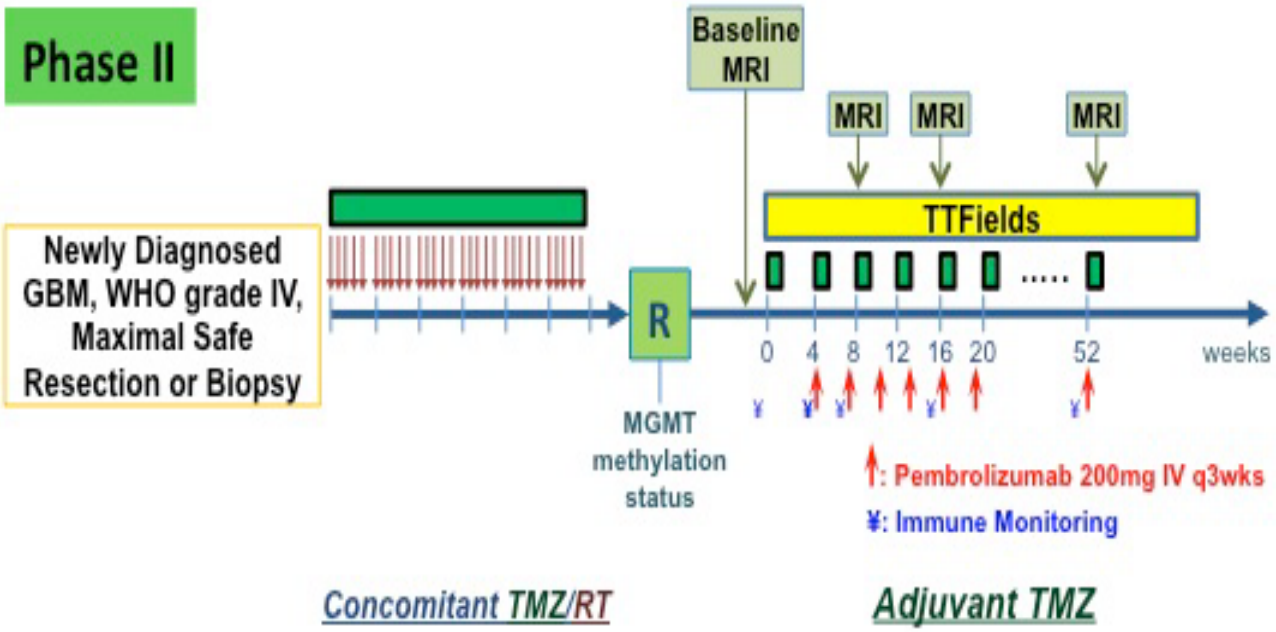
Glossary of Abbreviations

AE	Adverse Event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
ANOVA	Analysis of Variance
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BUN	Blood Urea Nitrogen
CBC	Complete blood count
CFR	Code of Federal Regulations

CMP	Comprehensive Metabolic Panel
CNS	Central nervous system
Cr	Serum Creatinine
CR	Complete Response
CRF	Case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DISC	Data Integrity and Safety Committee
ECHO	Echocardiography
ECI	Event of clinical interest
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme-Linked Immunoabsorbent Assay
EKG	Electrocardiogram
FDA	Food and Drug Administration
FLAIR	Fluid Attenuated Inversion Recovery
FWA	Federal wide assurance
GBM	Glioblastoma multiforme
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practice
HIV	Human Immunodeficiency Virus
IHC	Immunohistochemistry
IND	Investigational New Drug
INR	International normalized ratio
irAE	Immune-related adverse event
IRB	Institutional Review Board
IULN	Institutional upper limits of normal
IV	Intravenous
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NIH	National Institutes of Health
NSCLC	Non-small cell lung cancer
OHRP	Office of Human Research Protections
OS	Overall survival
PFS	Progression-free survival
PI	Principal Investigator
PT	Prothrombin time
PTT	Partial thromboplastic time
iRANO	immunotherapy Response Assessment in Neuro-Oncology
RN	Registered Nurse
RR	Radiographic Response
RT	Radiation Therapy

RTOG	Radiation Therapy Oncology Group
SAE	Serious adverse event
SD	Stable Disease
StD	Standard Deviation
SQ	Subcutaneous
SUSAR	Suspected unexpected serious adverse reaction
TIL	Tumor-infiltrating lymphocytes
TMZ	Temozolomide
TSH	Thyroid stimulating hormone
TTFields	Tumor Treating Electric Fields
UF	University of Florida
UPN	Unique patient number
WBI	Whole Body Irradiation

SCHEMA



R: Registration.

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1. PROTOCOL SYNOPSIS

Title:	Phase 2, Single Arm, Historically Controlled Study Testing The Safety and Efficacy of Adjuvant Temozolomide plus **TTFields (Optune®)** plus Pembrolizumab in Patients with Newly Diagnosed Glioblastoma (2-THE-TOP)
Drug/Device	Pembrolizumab, temozolomide, and **TTFields (Optune®)**
Study Objectives:	To determine whether the addition of pembrolizumab to the combination of **TTFields (Optune®)** and adjuvant temozolomide (Triple Combination) increases progression-free survival (PFS) in patients with newly diagnosed glioblastoma (GBM) as compared to historical control data in EF-14 study.
Study Design:	Open-labeled, historically controlled
Study Hypothesis:	The hypothesis of this study is that the addition of pembrolizumab to the combination of **TTFields (Optune®)** and adjuvant temozolomide (TMZ) will significantly increase the PFS of newly diagnosed GBM patients compared to a historical control.
Sample Size:	24 patients with newly diagnosed GBM
Study Population:	Patients 18 years and older with pathologic diagnosis of GBM WHO Grade IV who have undergone maximal resection or biopsy followed by radiation therapy with concomitant temozolomide (Stupp protocol) [1]
Primary Endpoint:	To determine whether the triple combination of pembrolizumab when added to **TTFields (Optune®)** and adjuvant temozolomide increases PFS in patients with newly diagnosed GBM as compared to historical control data in the EF-14 study.
Secondary Endpoint:	1. To determine toxicity and tolerability of the triple combination in newly diagnosed GBM patients 2. To determine overall survival (OS) and radiographic response (RR) of the triple combination in newly diagnosed GBM as compared to historical data in EF-14 study 3. To determine whether pembrolizumab augments TTFields-initiated glioma-specific immune reaction.
Exploratory endpoints:	1. Metabolomics signature of immune activation by TTFields and TTFields + pembrolizumab in serum and urine. 2. To determine if mutation burden, when available from standard of care genomics analysis, in primary tumor samples correlates with response to pembrolizumab in the presence of TTFields. 3. To determine if intratumoral expression of PD-L1 correlates with response to pembrolizumab in the presence of TTFields.
Sponsor:	Novocure Ltd. POB 15022 MATAM Center Haifa, 31905, Israel

2. BACKGROUND AND RATIONALE

2.1 Glioblastoma Multiforme

Glioblastoma is the most common primary malignant neoplasm of the central nervous system in adults with a median overall survival of 14.6 months and a 2 year survival rate of 28% with standard chemoradiation [2]. Tumor-treating fields are a novel loco-regional anti-mitotic treatment modality by interfering with formation of the spindle structure and other macromolecules during cell division [3]. In the planned interim analysis of 315 patients of the phase 3 EF-14 trial in newly diagnosed GBM comparing TTFields plus temozolomide to temozolomide alone adjuvant therapy, the addition of TTFields to adjuvant temozolomide significantly increased progression-free survival (PFS) (7.1 vs. 4.0 months; HR 0.62, p=0.001) and OS (20.5 vs. 15.6 months; HR 0.64; p=0.004) compared to temozolomide alone [4]. In the final analysis of all 695 patients, PFS for the combination vs. temozolomide alone was 6.7 vs. 4.0 months (hazard ratio [HR], 0.63 [95%CI 0.52, 0.76] P=0.00005) and median survival for the combination vs. temozolomide alone was 20.9 vs. 16 months (HR 0.63 [CI 0.53, 0.76] P=0.00006) [5]. These data establish TTFields plus adjuvant temozolomide as a new standard treatment for newly diagnosed GBM.

2.2 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 [6]. 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 [6, 7]. In addition, low frequency pulsed electric fields have been claimed to stimulate bone growth and accelerate fracture healing [8]. 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 [9]. 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 [10].

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 [9]. 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 [11]) and cell rotation [12, 13]. 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 [14].

2.3 Novocure's Tumor Treating Electric Fields (TTFields)

Novocure has shown [15] 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 [16]. 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 [17, 18] or indirectly [19-21] with microtubule polymerization (e.g., paclitaxel).

2.4 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 [3, 4, 22]. 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. The patient must learn to change and recharge depleted service batteries and to connect 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 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 [22]. 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.

2.5 Effect of Optune on Newly Diagnosed GBM patients - A Phase 3 Study

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 [4].

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 [4].

2.6 Immune Dysregulation

Recent work has collectively demonstrated striking immune dysregulation in patients with GBM, including T cell lymphopenia and anergy, cytokine dysregulation, increased regulatory T cell (Treg) populations, and NK cell dysfunction, among others, which reflect immunologic compromise and functional impairment [23, 24]. However, a growing list of potential tumor antigens has been identified, suggesting that tumor cell-specific recognition by immune cells may be biologically relevant and therapeutically exploitable [25]. Therefore, given the immune dysfunction characterized in GBM patients, approaches that potentiate the anti-glioma immune response are particularly exciting. A priori, because the immune system has evolved to recognize a tremendous diversity of antigenic epitopes in vertebrates, immune-potentiating efforts may be especially effective at targeting the heterogeneity that defines GBM. Recently, the success of antibody-based therapies targeting immune checkpoints has generated field-wide enthusiasm that biological therapies may be broadly effect across many cancer types, including GBM [26-31]. Of these agents, those targeting the negative regulator proteins CTLA-4 and PD-1/PD-L1 have shown clinical efficacy but have not been studied widely in newly diagnosed GBM patients.

2.7 Pembrolizumab - Keytruda

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades [32]. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue and favorable prognosis in various malignancies [33-37]. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector

T-cells/Foxp3+ regulatory T-cells seem to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4, which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [38, 39]. The structure of murine PD-1 has been resolved [40]. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70, which are involved in the CD3 T-cell signaling cascade [38, 41-43]. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins [44, 45]. PD-1 has been shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, Tregs and Natural Killer cells [46, 47]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [48]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [44, 49-51]. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues [44]. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma [52]. This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Keytruda™ (pembrolizumab) has recently been approved in the United States for the treatment of patients with advanced and metastatic non-small cell lung cancer, recurrent or metastatic head and neck squamous cell carcinoma, locally advanced urothelial carcinoma, classical Hodgkin lymphoma, unresectable or metastatic melanoma or melanoma progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor, and any solid tumor with high microsatellite instability or mismatch repair deficiency.

2.8 Rationale for Pembrolizumab Dosing

An open-label Phase I trial was conducted to evaluate the safety and clinical activity of single agent pembrolizumab [53]. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (Q2W) in subjects with advanced solid tumors. All three-dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of pembrolizumab showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg Q2W). No MTD was identified during the study date. Recent data from other clinical studies within the pembrolizumab program has shown that a lower dose of pembrolizumab and a less frequent schedule may be sufficient for target engagement and clinical activity [27, 29, 54].

PK data analysis of pembrolizumab administered Q2W and Q3W showed slow systemic clearance, limited volume of distribution, and a long half-life [55]. Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days) [56, 57]. This early PK and pharmacodynamic data provides scientific rationale for testing a Q2W and Q3W dosing schedule.

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.58, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights [58]. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication [57]. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg Q3W body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for pembrolizumab in the melanoma indication. The exposure margins were based on the notion of similar efficacy and safety in melanoma at 10 mg/kg Q3W vs. the dose regimen of 200 mg Q3W (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications [28, 55, 58]. Therefore, there are no anticipated changes in exposure between different indication settings.

An ongoing phase I and II study testing the safety and efficacy of pembrolizumab and laser ablation in recurrent high-grade gliomas (FDA IND 1250999; WUTSL IRB 201501072; PI: Jian Campian, M.D., Ph.D.) has completed the phase I trial with 9 patients in 3 dose cohorts. No dose limiting toxicity was observed. Pembrolizumab 200 mg IV Q3W (i.e. the highest dose in the study) was generally well-tolerated. One durable complete response and 4 patients with prolonged stable disease (range 5-21 months) were observed.

2.9 Study Rationale

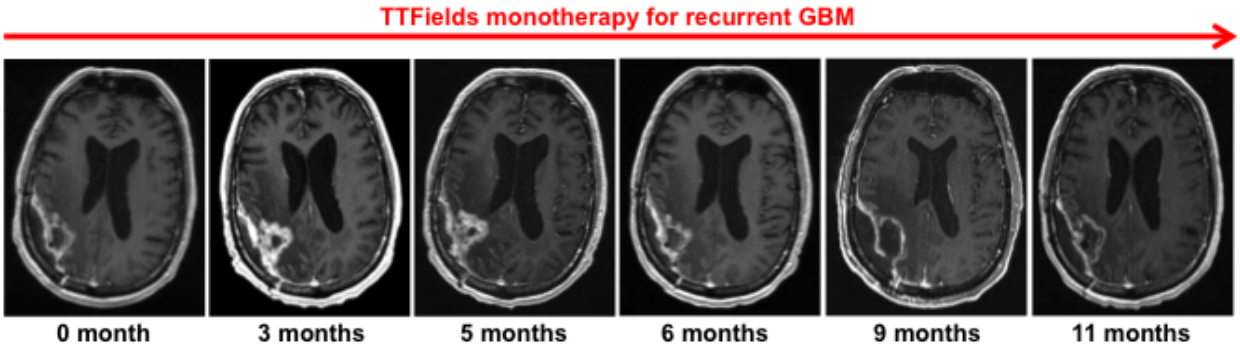
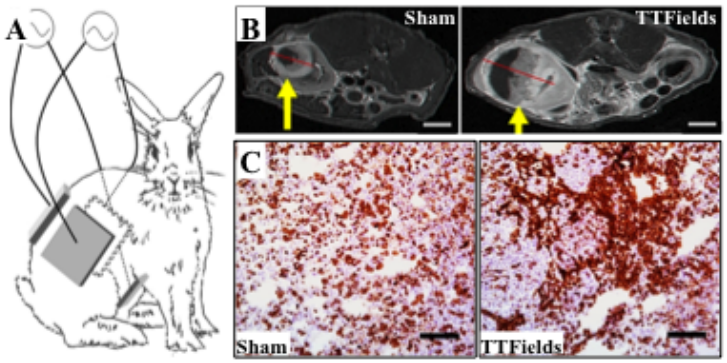


Figure 1: Pseudoprogression induced by TTFields. A representative set of brain MRI is presented. A 72 year old patient with non-methylated MGMT promoter , EGFRvIII-negative GBM developed recurrent tumor after 4 cycles of maintenance temozolomide. The patient was subsequently treated with TTFields alone. Increases in enhancement and FLAIR signal were noted 3 months after the start of TTFields. Perfusion showed low rCBV and patient was minimally symptomatic. TTFields was continued. Both enhancement and FLAIR subsequently demonstrated gradual reduction over the next 8 months.



D Lymphocyte infiltration in VX-2 tumors

Treatment	CD4	CD8	CD45	CD19
Sham	2.0 ± 0.8	0.8 ± 0.5	2.0 ± 0.8	0
TTFields	3.4 ± 0.9	1.6 ± 0.5	3.6 ± 0.5	0

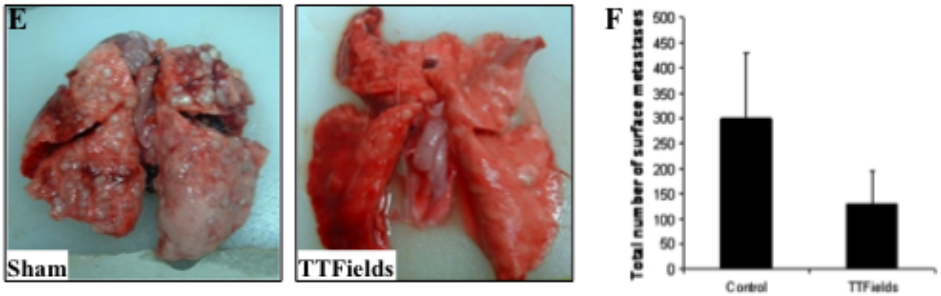


Figure 2: **TTFields induce an immune-mediated anti-tumor effect in a VX-2 carcinoma model in adult New Zealand white rabbits.** (Adapted from Kirson, et al, *Clin. Exp. Met*, 2009; 26(7):633-640)
(**A**) TTFields vs. sham treatment applied to the abdomen of adult New Zealand white rabbits implanted with VX-2 tumor cells in the kidney capsule. Treatment was initiated on day 12 from implantation of the kidney tumor. (**B**) T1 weighted, gadolinium-contrasted MR images of the maximal cross-sectional area (red line) of a TTFields vs. sham treated kidney VX-2 tumor (yellow arrows). TTFields treated tumor is larger compared to sham control. Scale bar is 1 cm. (**C**) Photographs of VX-2 tumor sections stained with CD4. Increased intra-tumoral infiltration of $CD4^+$ T cells in TTFields treated compared to sham treated tumor. *Scale bar* 100 µm. (**D**) The average number of intra-tumoral infiltration of hematologic cells and lymphocyte subsets in TTFields vs. sham treated tumors. (**E**) Photographs of lungs removed from same rabbits treated with TTFields or sham. (**F**) The average total number (±SD) of surface lung metastases in abdominal sham vs. TTFields treated rabbits. TTFields treatment to the abdominal VX-2 tumor resulted in a reduction in lung metastasis compared to the sham treated, indicating that TTFields may have induced systemic anti-tumor immunity.

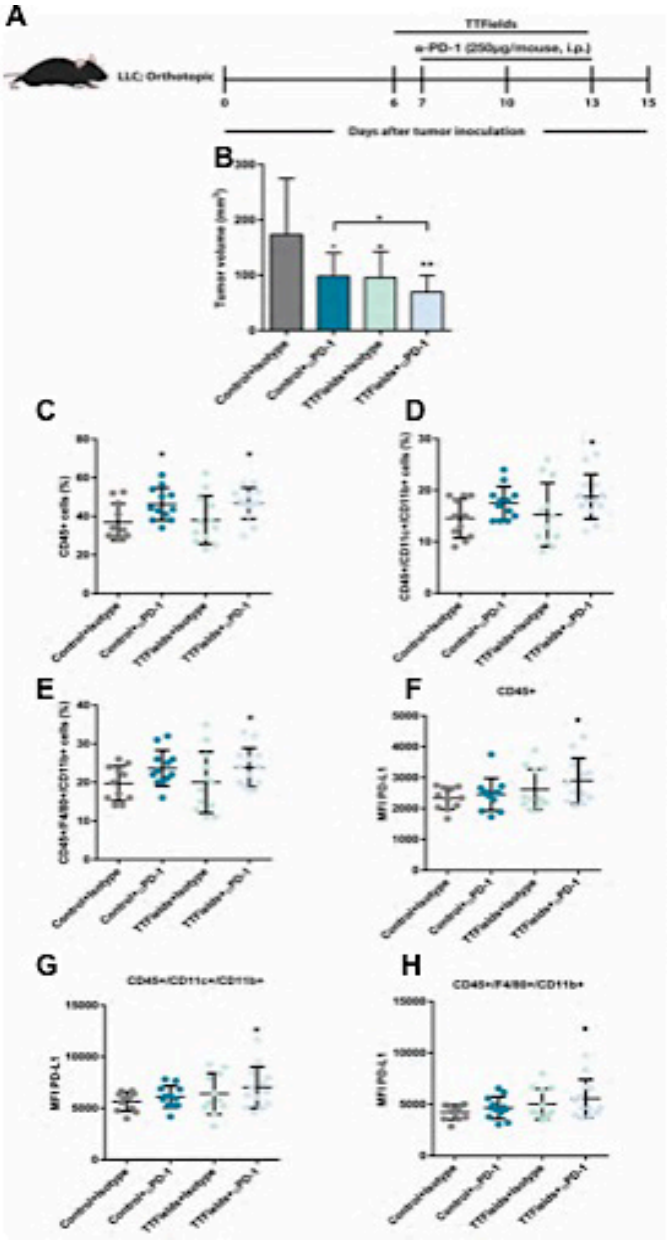


Figure 3. TTFields in Combination With Anti-PD-1 Are Therapeutically Effective in Vivo. (*Adapted from Moshe et al. Poster AAI 2016*)

10-12-week-old male C57Bl/6 mice were injected directly into the lungs with Lewis lung carcinoma (LLC-1; $3x10^5$ cells) (**A**). TTFields was initiated 6 days afterwards and maintained for 7 days. Mice received an I.P. injection of anti-PD-1 (αPD-1; RMP1- 14; 250 μg) or Rat IgG2a (2A3; 250 μg) (Bioxcell). The combination of TTFields and anti-PD-1 resulted in a decrease in tumor volume as compared to control and anti-PD-1 alone (**B**). The percentages (**C-E**) and PD-L1 expression levels (**F-H**) of tumor- infiltrating CD45+ cells (**C, F**), dendritic cells (CD11b+/CD11c+) (**D, G**), and macrophages (CD11b+/F4/80+) (**E, H**) were measured. CD45+ and specifically CD11b+/CD11c+ and CD11b+/F4/80+ cells from tumor in the combined treatment group exhibited both higher tumor infiltration and elevated PD-L1 expression, as compared to infiltrating immune cell in the control group. (n=13-14 mice in each group). Mean ± SD; **P*<.05, ***P*<.01 from control group, student's t-test.

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 **Fig.1** and unpublished data) [4, 22]. These observations suggest a possibility that TTFields may induce an inflammatory reaction in the tumor. In 2 separate immunocompetent animal models of cancer including 1) syngeneic mice injected with the mouse melanoma cell line B16F10, and 2) New Zealand white rabbits implanted with VX-2 carcinoma within the renal capsule, TTFields therapy to the primary tumors in the abdomen significantly reduced growth of distant lung metastasis, suggesting a systemic immune effect of TTFields. Within these primary tumors, TTFields induced a considerable increase in peri- and intra-tumoral immune cell infiltration, including both $CD4^+$ and $CD8^+$ lymphocytes, compared to sham-treated control tumors [59] (**Fig. 2**). Although the mechanism remains unclear, it is presumably through its anti-mitotic activities causing

immunogenic death of cancer cells, leading to an increase in cancer-associated antigen or neoantigen presentation and immune activation. These results also indicate that proliferating immune cells recruited to tumors were not susceptible to the anti-mitotic effect of the intermediate electrical field frequency of 100-200kHz used in TTFields. In fact, emerging data suggest that there appears to be an inverse correlation between cell size and intracellular electrical properties and the field frequency to which they are susceptible (data not shown).

In addition, recent data indicate that TTFields may improve immunotherapy such as immune checkpoint inhibitors. In the Lewis lung carcinoma models in mice, the combination of TTFields and an anti-PD-1 monoclonal antibody resulted in improved tumor control compared to TTFields alone or anti-PD-1 antibody alone (**Fig. 3**). The improved tumor control by the combination was associated with higher tumor infiltration of various immune cells, especially antigen presenting cells and elevated expression of PD-L1 (**Fig. 3**).

Taken together, these data support the notion that the early edema and delayed responses of GBM to TTFields may have an immunological basis and could be augmented if combined with an immune modulator. In this protocol, we propose to combine **TTFields** with **pembrolizumab** during the adjuvant **temozolomide** treatment phase for newly diagnosed GBM. We hypothesize that TTFields combined with temozolomide elicit anti-mitotic effects on proliferative cancer cells and also significantly augment recruitment of immune effector cells specific for glioma cells to the tumor microenvironment where pembrolizumab, a PD1 inhibitor, further potentiates the glioma-specific immune reaction to achieve a synergistic therapeutic effect.

2.10 Correlative Study Background

Level of expression of PD-L1 on tumor cells has been associated with response to anti-PD-1/PD-L1 checkpoint blockade therapy, although the association is not absolute [60]. Therefore, the degree of PD-L1 expression will be determined on tumor using IHC. RNA sequencing (RNAseq) can also be performed, if necessary, to determine if relative gene expression levels can serve as an alternative predictive biomarker as well as to determine the relative expression levels of other immune checkpoint pathways or immune responsive pathways (i.e. interferon-related genes) that may be associated with resistance or response to therapy.

Mutational burden has also been associated with response to checkpoint blockade therapy. GBM is generally considered to have low mutation burden compared to other extracranial tumors [61]. However recent study suggests that TTFields may also target DNA repair mechanisms [62], which potentially could increase mutation burden in treated tumors over time and render them more sensitive to checkpoint inhibitors. Tumor mutational load will be assessed by DNA sequencing and obtained from routine clinical Foundation One or internal analysis on pre-treatment tumor specimens.

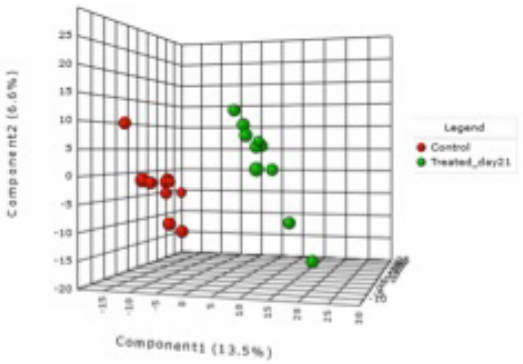


Figure 4: Changes in NMR signatures of serum immunometabolites in response to PD-1 inhibitor treatment. C57BL/6 mice orthotopically implanted GL261 tumors were treated with PD-1 antibody and sera were obtained before and 21 days after treatment for NMR spectroscopy.

The glioma-specific immune response, molecular, and genomic analysis will be assessed in peripheral blood. Pre- and post-treatment peripheral blood will be assessed by flow cytometry for markers of T cell activation and anergy as well as cytokine profiles. Furthermore, serum or plasma will be assessed for the presence and level of soluble immune checkpoint molecules, cytokines, and chemokines using commercially-available multiplex immunoassays.

Recently immunometabolic markers of specific immune activation can be detected in the serum or urine and have been suggested as a non-invasive method to monitor vaccination reactions in real time [63]. We have applied this concept to monitoring the response of mice bearing a syngeneic GBM model to a PD-1 inhibitor and detected a clear shift of certain immunometabolic markers in both serum (**Fig. 4**) and urine (not shown). We will apply this technique to explore its utility in detecting and monitoring immune response to TTFields and to TTFields + pembrolizumab combination.

3. OBJECTIVES

This prospective, single-arm, open-label study will be conducted in patients with newly diagnosed GBM who have completed standard of care radiation therapy together with concomitant TMZ. In this protocol, we propose to combine **TTFields** (utilizing the Optune System**)** with **pembrolizumab** during the adjuvant **TMZ** treatment phase for newly diagnosed GBM. We hypothesize that TTFields combined with TMZ will elicit anti-mitotic effects on proliferative cancer cells and also significantly augment recruitment of immune effector cells specific for glioma cells to the tumor microenvironment where pembrolizumab, a PD1 inhibitor, further potentiates the glioma-specific immune reaction to achieve a synergistic therapeutic effect.

3.1 Primary Objectives

To determine whether the triple combination (TTFields, temozolomide, pembrolizumab) increases PFS in patients with newly diagnosed GBM as compared to historical control data (TTFields, temozolomide) in the phase 3 registration EF-14 study.

3.2 Secondary Objectives

1. To determine toxicity and tolerability of the triple combination in newly diagnosed GBM patients
2. To determine OS and RR of the triple combination in newly diagnosed GBM as compared to historical data in the EF14 study.
3. To determine whether pembrolizumab augments TTFields-initiated glioma-specific immune reaction.

3.3 Exploratory Objectives

1. Metabolomics signature of immune activation by TTFields and TTFields + pembrolizumab in serum and urine.
2. To determine if mutation burden, when available from standard of care genomics analysis, in primary tumor samples correlates with response to pembrolizumab in the presence of TTFields.
3. To determine if intratumoral expression of PD-L1 correlates with response to pembrolizumab in the presence of TTFields.

4. PATIENT SELECTION

4.1 Inclusion Criteria

1) Histologic confirmation of glioblastoma, WHO Grade IV (GBM variants are allowed).

2) MGMT methylation status if available (indeterminate methylation status will be considered unmethylated).

3) Karnofsky performance status (KPS) ≥70%.

4) Patients must be at least 18 years of age.

5) Received maximal safe resection (biopsy only allowed) and radiotherapy concomitant with temozolomide:

   a. Gliadel wafers placement at the time of surgical resection is allowed.
   b. Any additional treatment directed at GBM will be considered exclusionary.
   c. Minimum dose for concomitant radiotherapy is 40 Gy.

6) Candidate for adjuvant high dose temozolomide and Optune therapy.

7) Life expectancy of at least 3 months.

8) Adequate bone marrow and organ function as defined below:

   a. ANC ≥ 1,500/mcL
   b. Platelets ≥ 100,000/mcL
   c. Hemoglobin ≥ 9 g/dL or ≥ 5.6 mmol/L (transfusion is allowed)
   d. Serum creatinine ≤ 1.5 x IULN OR creatinine clearance by Cockcroft-Gault ≥ 60 mL/min for patients with serum creatinine > 1.5 x IULN
   e. Serum total bilirubin ≤ 1.5 x IULN OR direct bilirubin ≤ IULN for patients with total bilirubin > 1.5 x IULN
   f. AST (SGOT) and ALT (SGPT) ≤ 3 x IULN

9) Participants of childbearing age must use effective contraception:

   - 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. 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. Refer to Appendix D for guidance on highly effective contraceptive methods.
   - WOCBP include any woman who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or oophorectomy) or who is not post-menopausal. Post-menopause is defined as:

- Amenorrhea that has lasted for ≥ 12 consecutive months without another cause, or
- 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.
- Males with female partners of childbearing 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.

10) Ability of the patient to understand and willingness to sign an IRB approved written informed consent document

11) Steroid dose equivalent to dexamethasone dose of ≤ 4mg daily at the time of starting adjuvant treatment

12) Optune and temozolomide treatment start date will be at least 4 weeks but not more than 6 weeks from the later of last dose of concomitant temozolomide or radiotherapy. Although Optune and temozolomide should be started simultaneously, it is not required as long as both are started within this time frame.

4.2 Exclusion Criteria

1. Prior treatment with anti-angiogenic agents including bevacizumab.

2. History of other malignancy that, in the primary oncologist's estimation, has a higher risk of recurrence or death than the study-related cancer at the time of study participation.

3. Prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways).

4. Progressive disease (according to RANO criteria). Advanced imaging is allowed to further investigate suspected pseudoprogression if deemed necessary.

5. Actively participating in another clinical treatment trial intended to treat GBM.

6. Multifocal gliomas defined as distinct tumors that do not have overlapping T2/FLAIR signal.

7. Presence of leptomeningeal metastases.

8. Implanted pacemaker, programmable shunts, defibrillator, deep brain stimulator, other implanted electronic devices in the brain, or documented clinically significant arrhythmias.

9. Tumor is entirely located in the infra-tentorial region.

10. History of hypersensitivity reactions or allergies to hydrogels and/or compounds of similar chemical or biologic composition to Temozolomide and Pembrolizumab.

11. Steroid dose equivalent to > 4 mg dexamethasone at the time of starting adjuvant therapy.

12. History of immunodeficiency or is receiving any form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment (with the exception of daily dexamethasone ≤ 4 mg).

13. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, uncontrolled hypertension, or psychiatric illness/social situations that would limit compliance with study requirements.

14. History of active autoimmune disease requiring systemic treatment within the past 2 years (i.e. with use of disease modifying agents, corticosteroids, or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

15. History of (non-infectious) pneumonitis that required steroids or current pneumonitis.

16. Pregnant and/or breastfeeding. Patient must have a negative serum or urine pregnancy test within 72 hours of study entry.

17. Females or males of childbearing potential who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for at least 24 weeks after the last dose of study drug.

18. Known active hepatitis B (e.g., HBsAg reactive) or hepatitis C (e.g., HCV RNA [qualitative] is detected) infection.

19. Known history of active TB (bacillus tuberculosis).

20. Known history of HIV (HIV 1/2 antibodies).

4.3 Inclusion of Women and Minorities

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

5. SUBJECT RECRUITMENT AND REGISTRATION PROCEDURES

Potential subjects will be recruited to this study from the outpatient clinic after confirmation of tumor pathology when patients are seen to discuss diagnosis, prognosis and treatment options which may include participation in a clinical trial. If a patient is a potential candidate and interested in more information about a specific trial, the Physician-Investigator and/or the research coordinator will discuss the trial further as part of the consent process. All potential subjects will be seen by a study investigator and will be required to sign a written informed consent prior to

being registered on this protocol. Every effort will be made to answer questions raised by the patient and their family or advocate regarding the protocol and alternative therapies prior to asking the patient to sign the consent form. Subjects will be registered after the completion of radiation and concomitant TMZ. MGMT status is not required to sign consent but should be confirmed prior to study registration if feasible (indeterminate methylation status will be considered unmethylated). Both men and women and members of all races and ethnic groups are eligible for this trial.

5.1 Subject Registration in the University of Florida Cancer Center Database

All study subjects will be registered through OnCore at the University of Florida. Each subject will be identified with a unique patient number (UPN) for this study and by first, middle, and last initials. If the subject has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this UPN on the appropriate CRFs.

6. TREATMENT PLAN

Patients with newly-diagnosed GBM who undergo maximal safe resection (biopsy alone is eligible) followed by chemoradiation consisting of concomitant TMZ 75mg/m^2 daily and RT with minimal RT dose of 40gy will be eligible for this trial. MGMT methylation status will be recorded.

Four weeks (+7/-14 days) after completing chemoradiation, patients will undergo baseline standard of care MRI. Four to six weeks after finishing chemoradiation, patients will start monthly cycles of adjuvant TMZ. A minimum of 6 and maximum of 12 cycles of adjuvant TMZ will be given depending on tolerability and toxicity.

Treatment with Optune will start at approximately the same time as the first cycle of adjuvant TMZ and continue until second disease progression or a maximum of 2 years. Urine specimens will be collected as well as peripheral blood mononuclear cells (PBMC) will be obtained and cryo-preserved at time points specified. No sooner than 3 weeks after starting Optune therapy, patients will begin open-label treatment with pembrolizumab 200mg IV every 3 weeks until first disease progression or unacceptable toxicities or 2 years, whichever comes first.

At first progression, patients will be allowed to continue with Optune therapy combined with any other therapy, which may include pembrolizumab, per standard of care at the discretion of the treating physician. Surgical resection or biopsy of first recurrent tumor for confirmation of recurrence is allowed within the protocol.

For correlative studies, flash frozen tumor tissues, where available, will be used to prime dendritic cells. Intratumoral PD-1 expression will also be determined by IHC using FFPE slides. Research blood will be drawn prior to starting adjuvant therapy and within approximately 1 week before the first dose of pembrolizumab. Subsequently research blood will be drawn prior to the second dose of pembrolizumab, and at approximately 2-, 4-, 6-, 12-, and 24-months (+/- 2 weeks) post-pembrolizumab dosing. Urine samples will be obtained at the same time points as research blood. If there is evidence of progression, however, a final research blood draw and urine sample will be obtained within 30 (+/- 7) days of progression and no further research blood draw and urine specimen will be performed (unless the patient continues on Optune therapy to second progression as detailed in the paragraph above). Research blood will also be collected before resection or

biopsy, when possible. Mutation burden, when available, will be obtained from next gene sequencing analysis per standard of care.

6.1 Treatment with Optune

Patients will undergo 24-months of planned treatment with Optune therapy. Therapy will be given as per standard of care (see Section 6.1.1 for recommended use of Optune). 3D mapping and placement maps of recurrent tumors will be completed by the treating physician or Novocure. All device support services (including patients' and families' education of device operation, tips about scalp shaving and array placement, downloading and processing of technical and compliance data, and troubleshooting device malfunction) will be provided by device support specialists employed by Novocure. The goal of treatment compliance for this study is wearing the system for an average of no less than 75% of the time over a 4-week period for as many months as possible. Compliance reports with Optune will be obtained by Novocure's DSS per usual procedures. If a patient's compliance is below 50% over the first and second 4-week periods consecutively from the start of treatment, the patient will be trained on the importance of compliance and any barriers to compliance will be addressed. If a patient's compliance is still below 50% in the third 4-week period, the patient will be removed from study and that subject should be considered non-evaluable for survival analysis and should be replaced. This subject however should remain evaluable for the toxicity and tolerability endpoints as per end of treatment requirement detailed in section 6.6. At the discretion of treating investigators, patients can have an additional treatment break during each 4 week period of no more than 4 days as needed for personal reasons as long as it is estimated that the overall compliance for any 4 week period does not drop below 75% because of the additional break.

6.1.1 Recommendations for Use of Optune

All patients 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. Optune is programmed by Novocure to deliver 200 kHz TTFields in two sequential, perpendicular field directions at a maximal intensity of 707mARMS. 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 6.1.2.

There will be no dose adjustments to the device for adverse events.

6.1.2 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% Clobetasol) 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.

6.1.3 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

6.2 Pembrolizumab Administration

Pembrolizumab 200 mg will be given intravenously every 3 weeks (+/- 4 days) beginning no sooner than 3 weeks after the start of Optune. Pembrolizumab will be given intravenously over the course of approximately 30 minutes on an outpatient basis. For subjects who receive pembrolizumab alone after the completion of TMZ or due to TMZ intolerance, pembrolizumab infusions may be administered per the treatment plan if absolute neutrophil count (ANC) is $\geq 1.0 \times 10^9$/L, and the platelet count is $\geq 50 \times 10^9$/L, and if it is determined by the treating physician that the cytopenia was unlikely to be related to pembrolizumab treatment.

6.2.1 Premedication Administration of Pembrolizumab

No premeditations are required, but antiemetics may be given as per institutional practice if needed.

6.3 Temozolomide Administration

Patients will begin treatment with adjuvant TMZ at least 4 weeks but no more than 6 weeks from last dose of concomitant temozolomide or radiation therapy (the latter of the two). A minimum of 6 and maximum of 12 cycles of adjuvant TMZ will be given depending on tolerability and toxicity.

6.3.1 Adjuvant Temozolomide Treatment

- **Adjuvant Phase Cycle 1 (4-6 weeks after chemoradiation):** Dosage in Cycle 1 will be 150 mg/m^2 PO daily for 5 consecutive days per 28 (+7) day cycle.

- **Cycle 2 (up to Cycle 12):** At the start of Cycle 2, the dose of TMZ will be increased to 200 mg/m^2 PO daily for 5 consecutive days per 28 (+7) day cycle, if the CTC non-hematologic toxicity for Cycle one 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 TMZ dose will remain at 200 mg/m^2 PO daily for 5 consecutive days per 28 (+7) day cycle for each subsequent cycle except if toxicity occurs (see section 8.1). If the dose is not increased at Cycle 2 due to toxicity, escalation should not occur in subsequent cycles; however, it is appropriate to decrease to cycle 1 dose in cycles 2-12 if toxicity occurs.

6.4 Follow-Up Evaluations During The 12 Cycles of Adjuvant TMZ

All patients will be seen before Cycle 1 of TMZ, before starting the pembrolizumab, and every 3 weeks (+/-4 days) before each subsequent pembrolizumab dose at an outpatient clinic until they complete all 12 cycles of adjuvant TMZ or discontinue TMZ due to toxicity or first progression. Optune compliance reports will be collected remotely or in the clinic setting approximately every 4 (+/- 1) weeks. Contiguous data will be sufficient for Optune compliance reporting, per SOC for the Optune Device.

At each visit the following will be performed except otherwise specified:

- Physical exam and performance status (KPS)
- Blood exams
    - CBC with differential - within 3 days prior to starting each cycle of temozolomide
    - Complete Metabolic Panel - prior to each cycle of pembrolizumab and prior to starting each cycle of temozolomide (+/- 7 days)
    - PT/PTT, INR – for patients receiving relevant anti-coagulants
    - TSH, Free T4 – prior to the first infusion of pembrolizumab and then prior to every other pembrolizumab infusion thereafter
- Study lab as specified
- Steroid dose
- Concomitant Medications
- Record of Adverse Events
- TTF compliance report (only every 4 +/- 1 weeks)

An MRI will be performed every 2 months (+/- 7 days) following the baseline MRI until second progression (when study treatment will be terminated). In the case of clinical progression an unscheduled MRI will be obtained within 1 week of the investigator becoming aware of the clinical progression. Contrast agent type and dose will be kept constant for each patient between scans. Disease will be assessed as per iRANO criteria [64] [65] with allowance for potential immune mediated effects at first appearance. No additional MRIs will be required by the study after second progression. Medical follow-up will continue for 30 (+/- 7) days after treatment termination in

order to capture treatment related toxicities. After this visit, mortality will be assessed based on telephone interviews with the patients or the patients' caregivers every 3 months.

6.5 Evaluation After Adjuvant TMZ Until Treatment Termination

After 12 cycles of adjuvant TMZ, patients will be seen and examined before every three pembrolizumab doses. Pembrolizumab and Optune can continue up to 2 years. Optune compliance reports will be collected remotely or in the clinic setting every 4 (+/- 1) weeks. Brain MRI and quality of life assessment will be performed every 2 months (+/- 7 days) until study termination or second progression, whichever comes first. In case of clinical progression an MRI will be performed within a week of the treating physician being made aware. Contrast agent type and dose will be kept constant for each patient between scans. Disease will be assessed as per iRANO criteria [64] [65]with allowance for potential immune mediated effects at first appearance.

At each visit the following will be performed except for otherwise noted:

- Physical exam and performance status (KPS)
- Blood exams
    - CBC with differential - within 3 days prior to starting each cycle of temozolomide
    - Complete Metabolic Panel - prior to each dose of pembrolizumab and prior to starting each cycle of temozolomide (+/- 7 days)
    - PT/PTT, INR – for patients receiving relevant anti-coagulants
    - TSH, Free T4 – prior to the first infusion of pembrolizumab and then prior to every other pembrolizumab infusion thereafter
- Study lab as specified
- Steroid dose
- Concomitant Medications
- Record of Adverse Events
- TTF compliance report (every 4 +/-1 weeks)

6.6 Post Treatment Evaluations

Treatment will continue according to the protocol until toxicity, second progression or 24 months (the earlier of the three). After treatment termination the patient will be followed every three months via phone call or collected from clinic notes for survival follow up until death or for 5 years, whichever occurs first.

Subjects will be evaluable for survival analysis if they have received Optune treatment, AND have received at least one dose of pembrolizumab, AND have received adjuvant temozolomide. Subjects that are not evaluable for survival analysis will be replaced.

7. CORRELATIVE STUDIES

7.1 Blood Specimens

7.1.1 Collection of Specimens

Sixty milliliters of blood will be collected into 6 green top tubes (with heparin) for isolation of PBMCs at the following time points:

- No more than two weeks prior to starting adjuvant therapy.
- No more than one week prior to the first dose of pembrolizumab.
- No more than one week prior to the second dose of pembrolizumab and then approximately 2-, 4-, 6-, 12-, and 24-months after the first dose of pembrolizumab.
- At the end-of-treatment with Pembrolizumab the research blood draw will be obtained at the next scheduled clinic visit. Clinic visits after treatment concludes shall be scheduled per standard care.
    - If study treatment is ended due to disease progression or toxicity prior to the 24 months study term, a final research blood draw will be obtained within 30 (+/- 7) days of progression and no further research blood draws will be performed (unless the patient continues on Optune therapy to second progression as detailed in section 6).
    - If the study treatment is ended at 24 months as stipulated by the protocol, the end-of-treatment sample will be obtained at the next SOC visit and at the time of progression.
- In the event of surgical resection or biopsy (refer to section 7.2.1), research labs will be collected as close to the date of surgery as possible, but not more than two weeks prior to surgery.

7.1.2 Handling of Specimens

One 10 mL vial will be used for isolation of plasma via centrifugation for cytokine analysis and NMR analysis for metabolic signatures of immune activation. The remaining vials will be used for isolation of peripheral mononuclear cells (PBMCs) using Ficoll and cryopreserved until ready for analysis per standard protocol [66, 67]. For the PBMC isolated from 40ml of blood obtained prior to the start of adjuvant therapy, all will be used to isolate monocytes per standard protocol [67]. The isolated monocytes will be incubated with IL-4 and GM-CSF to produce dendritic cells (DC)[67], which will then be cryopreserved for analysis of tumor-specific functional activity of peripheral blood immune cells. To determine the phenotype of peripheral blood immune cells, following the 4-week interval, cryopreserved PBMC will be thawed and stained with labeled antibodies to CD4, CD8, CD45, CCR7, CD27, CD28, and CD62, markers of T cell hypofunctionality (e.g. PD-1, CTLA-4, LAG-3, TIM3, FoxP3, and ICOS), as well as MDSC (e.g. CD11b, CD33, CD14, CD15, Lin, HLA-DR). A subset of our analysis will be a comparison of $CD8^+PD\text{-}1^+LAG\text{-}3^+$ T cells between before and after starting Optune and before and after starting pembrolizumab as this cell population has been shown to mark tumor-specific $CD8^+$ T cells [68]. Cells will be assessed by flow cytometry in the Preston Wells, Jr. Center for Brain Tumor Therapy or by single cell genomics analysis for a comparison between before and after starting Optune and before and after starting pembrolizumab.

7.1.3 Transport of Specimens

Blood samples collected should be transported to Dr. Rahman's lab to be processed at ambient temperature immediately after collection and processed immediately upon arrival. If a delay in

transport or processing of greater than 1 hour is anticipated, samples must be stored on ice. Samples should be processed within 4 hours of collection.

7.2 Tumor Tissue Specimens

7.2.1 Collection of Specimens

Any flash frozen tissue (from either surgical resection or biopsy) available after histopathologic diagnosis of GBM, that will be collected for research purposes, will be obtained for priming of DC. Fixed and paraffin embedded samples will be obtained from pathology or research tissue banks for IHC.

7.2.2 Handling of Specimens

Tissue and PBMC cryopreservation are per standard protocol. FFPE samples will be used to test for PD-L1 IHC to be performed in Dr. Rahman's lab or the ICBR core lab. Flash frozen tumor samples will be minced and lysed into tumor lysate or treated with RNA buffer for total RNA isolation for DC priming. Parts of tissue will also be subjected to genomics analysis of the tissue to study the tumor immune microenvironment and correlate these findings with changes in immune systems related to Optune and Optune plus pembrolizumab.

7.2.3 Transport of Specimens

FFPE samples will be transported at room temperature. Frozen samples will be transported on dry ice or in liquid nitrogen to Dr. Rahman's lab.

Contact persons: Alexandra Calinescu (Alexandra.Calinescu@neurosurgery.ufl.edu) or Nagheme Thomas (thomasn@ufl.edu)

Frozen samples will be kept at-80^{o}C until ready for processing.

7.3 Urine Specimens

7.3.1 Collection of Specimens

Approximately twenty milliliters of clean-catch urine will be collected into a sterile plastic cup prior to starting adjuvant therapy and within approximately 1 week before the first dose of pembrolizumab. Subsequently research urine will be collected prior to the second dose of pembrolizumab, and at approximately 2-, 4-, 6-, 12-, and 24-months (+/- 2 weeks) post-pembrolizumab. If study treatment is terminated due to disease progression or toxicity prior to the 24 months study term, a final urine sample will be obtained within 30 (+/- 7) days of progression.

7.3.2 Handling of Specimens

Urine samples will be stored at -80ºC until analysis. Samples will be analyzed using NMR technique to detect metabolomic signatures of immune activation by comparing the pre-adjuvant/Optune samples to the post-adjuvant/Optune, but pre-pembrolizumab samples. These samples will be compared to the post-pembrolizumab samples.

7.3.3 Transport of Specimens

Urine samples will be transported to the Rahman Lab on ice within 1 hour of collection.

8. ADVERSE REACTIONS AND DOSE DELAYS/DOSE MODIFICATIONS

8.1 Potential Adverse Events: Temozolomide

Treatment with TMZ commonly (≥10%) causes the following adverse reactions: alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, coordination abnormal, viral infection, amnesia, and insomnia.

Treatment with TMZ commonly (≥10%) causes the following adverse reactions: lymphopenia, thrombocytopenia, neutropenia and leukopenia.

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

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

Table 1: Temozolomide Dose Levels for Adjuvant Treatment

Dose Level	Dose (mg/m2/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 2: Temozolomide Dose Reduction or Discontinuation During Adjuvant Treatment

Toxicity	Reduce TMZ by 1 Dose Level[a]	Discontinue TMZ

Absolute Neutrophil Count	$<1.0 \times 10^9/L$	See footnote b
Platelet Count	$<50 \times 10^9/L$	See footnote b
CTC Non-hematological Toxicity (Except for alopecia, nausea, vomiting)	CTC Grade 3-4	See footnote b

a: TMZ dose levels are listed in table 1.
b: TMZ is to be discontinued if dose reduction to <100 mg/m2 is required or if the same Grade 3 non-hematological toxicity (except for alopecia, nausea, vomiting) recurs after dose reduction.
TMZ = temozolomide; CTC = Common Toxicity Criteria.

8.2 Potential Adverse Events: Pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in the Table 3 below.

Table 3: Pembrolizumab Dose Reduction or Discontinuation

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver

Increased bilirubin	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	enzyme value returned to baseline or is stable
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	• Monitor for signs and symptoms of thyroid disorders.
Nephritis with Renal dysfunction	Grade 2 or 3 Increased blood creatinine	Withhold	• Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 4 Increased blood creatinine	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for increase in lesion size or frequency during corticosteroid course. Resume in patients with complete or partial resolution (Grades 0 to 1) after corticosteroid taper. Permanently discontinue if no complete or partial resolution within 12 weeks of initiating
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		

				steroids or inability to reduce prednisone to 10 mg per day or less (or equivalent) within 12 weeks of initiating steroids.
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3, or 4	Permanently discontinue		
All other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on type and severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.

NOTE:

For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

8.2.1 Supportive Care Guidelines for Infusion Reactions

Pembrolizumab may cause severe or life-threatening reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

The table below shows treatment guidelines for patients who experience an infusion reaction associated with administration of pembrolizumab.

Table 4. Supportive Care Guidelines for Infusion Reactions

NCI CTCAE Grade	**Treatment**	**Premedication at subsequent dosing**
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids, antihistamines, NSAIDS, acetaminophen, Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of MK-3475 with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids, antihistamines, NSAIDS, acetaminophen, narcotics, oxygen, pressors, corticosteroids, epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing

Grade 4: Life-threatening; pressor or ventilator support indicated		
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

8.3 Additional Potential Risks

8.3.1 Disease progression

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

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

8.3.2 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 patients with any signs or symptoms of cerebral edema may need their steroid doses increased, treatment with an osmotic diuretic and/or an, anti-angiogenic, or surgical decompression as per standard practice. 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, pre-operative neurological impairment, and post-operative course prior to starting study treatment. Patients will be monitored throughout the course of the study.

Cerebral edema toxicity exception:

NCI CTCAE criteria categorize all cerebral edema as grade 4.

Cerebral edema, with or without mass effect, normally presents in glioblastoma patients as part of the disease process and can be exacerbated by standard of care chemotherapy and radiation. Furthermore, an effective anti-tumor immune response may involve inflammatory response and edema in infiltrative tumor cells. Therefore, cerebral edema, for purposes of this protocol, although ranked grade IV 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 Phlebotomy and/or Intravenous Catheter Insertion

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.

8.3.4 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.

8.3.5 Allergic Reactions to Contrast Agents

During the MRI, patients will be given the contrast agent gadolinium. 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 excluded from the study. Patients with mild allergies (i.e., rash only) will be pretreated with Tylenol and Benadryl prior to injection of the contrast agent.

8.3.6 Privacy/Confidentiality

Participation in research may result in a loss of privacy should there be a breach in confidentiality. All data will be coded to protect the patient's identity so that risks are minimized; 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, and/or OHRP and the study sponsor. Any publications resulting from this study will not use patient identifying data.

8.4 Women of Childbearing Potential

WOCBP include any woman who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation or oophorectomy) or who is not post-menopausal. Post-menopause is defined as:

- Amenorrhea that has lasted for ≥ 12 consecutive months without another cause, or

- 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.
- Males with female partners of childbearing 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.

WOCBP must use highly effective contraceptive methods as described in Appendix D.

9. REGULATORY AND REPORTING REQUIREMENTS

9.1 Federal Regulations and Good Clinical Practice

The study will be conducted in accordance with the protocol, the Declaration of Helsinki, FDA Regulations 21 CFR parts 50, 54, 56 and 312 (as applicable), HHS Regulations 45 CFR part 46 (as applicable), the International Conference on Harmonization guidelines for Good Clinical Practice (ICH E6), and applicable institutional, local and state requirements. All personnel involved in the conduct of this study have completed human subjects protection and any other applicable research training in accordance with local institutional requirements.

9.2 Institutional Review Board

The PI will obtain Institutional Review Board (IRB) approval of the protocol and protocol related documents including consent forms, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects, prior to initiating the study. The study may be initiated only after the Principal Investigator has received written and dated approval from the IRB. Amendments to the protocol must originate with the Protocol PI and be approved by the IRB prior to implementation.

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form in accordance with 21 CFR 56 and local policy prior to implementation except where necessary to eliminate apparent immediate hazard to the subject. If a change is made to eliminate an apparent immediate hazard, the PI must notify the IRB in accordance with local policy. The investigator will follow the requirements of the local IRB for periodic reporting of study progress, reporting of serious adverse events, unanticipated problems, and protocol deviations or violations, safety monitoring reports and study completion. Protocol exceptions must have approval from the IRB prior to implementation.

The Principal Investigator must obtain protocol re-approval as required by the IRB, but not less than once per year.

9.3 Compliance with Laws and Regulations

In addition to the regulations, guidance and requirements outlined in Section 9.1, all UF Health investigator-initiated trials, meeting the criteria of the FDAAA's applicable clinical trials, will be registered with ClinicalTrials.gov by the PI or assigned designee. All studies must be registered

no later than 21 days after enrollment of the first participant. The PI or designee will maintain the responsibility of updating trials registered with ClinicalTrials.gov; per the FDA's updating requirements of information must be updated at least every twelve months and the registry must be updated within thirty days of any changes in recruitment status or completion of the study.

9.4 Delegation of Investigator Responsibilities

The Principal Investigator will ensure that all persons assisting with the study are adequately informed about the protocol, any amendments to the protocol, the study treatments, and their study-related duties and functions. The Principal Investigator will maintain a list of Co-Investigators and other appropriately qualified persons to whom significant study-related duties have been delegated.

Study personnel involved in study conduct will be qualified by education, training, and experience to perform their respective tasks.

9.5 Subject Information and Informed Consent

Informed consent will be obtained and documented in accordance with 21 CFR 50 and the requirements of the IRB. The informed consent form must be written in a manner that is understandable to the subject population. Prior to its use, the informed consent form must be approved by the IRB.

The Principal Investigator or authorized key personnel will discuss with the potential subject the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Appropriate accommodations will be made available for potential subjects who cannot read or understand English or are visually impaired. Potential subjects will have the opportunity to contact the Principal investigator or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study.

Before conducting any study-specific procedures, the Principal Investigator must obtain written informed consent from the subject. The original informed consent form will be maintained with the subject's study records, and a copy of the informed consent form will be provided to the subject. The Principal Investigator is responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

9.6 Privacy/Confidentiality

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future

visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. After agreeing to participate, some patient identifiers will be collected, used and/or recorded, including, but not limited to name, date of birth, telephone number, address, dates from health records and a unique patient number. This information may be kept in patient binders as well as recorded in the respective eCRF. Images collected will be as de-identified as possible. Only approved study personnel and those included in the HIPAA Authorization will have access to identified, partially identified, and/or coded data. Data and specimens will be collected, stored and transported with consideration for subject privacy and confidentiality. Electronic records of subject data will be maintained in a password-protected computer. Access to electronic databases will be limited to the Principal Investigator and key study personnel. Data stored on portable memory devices will be de-identified.
Upon completion of the study, research records will be archived and handled per local policy.
Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

9.7 Protocol Amendments

All protocol amendments (including consent form changes and changes to other protocol documents) must be initiated by the PI and be approved by the IRB prior to implementation. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Principal Investigator must inform the IRB per local policy. Deviations from the approved protocol will be recorded and reported to the IRB per policy.

9.8 Case Report Forms

The Principal Investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs in accordance with the CTMS study calendar, using single data entry with a secure access account.

Electronic case report form (eCRF) will be utilized for this trial and must be completed for each included subject.

9.9 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all

reports and records necessary for the evaluation and reconstruction of the clinical research study.

The Principal Investigator will maintain study-related records in compliance with local record retention policies.

UF Health Cancer Center requires that all study documentation be maintained for at least 6 years from the date of final study publication. No study records may be destroyed without prior authorization from UF.

9.10 Conflict of Interest

The Principal Investigator and Sub-Investigators must comply with applicable federal, state, and local regulations regarding reporting and disclosure of conflict of interest. Conflicts of interest may arise from situations in which financial or other personal considerations have the potential to compromise or bias professional judgment and objectivity. Conflicts of interest include but are not limited to royalty or consulting fees, speaking honoraria, advisory board appointments, publicly-traded or privately-held equities, stock options, intellectual property, and gifts. Investigators and key personnel must provide financial disclosures prior to trial participation and as interests change and complete any applicable institutional conflict of interest documentation per local policy.

9.11 Adverse Event Definitions, Reporting and Documentation

9.11.1 Adverse Events (AEs)

Definition: An AE is any untoward medical occurrence associated with use of a drug, whether or not considered drug related. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug, whether or not related to use of the study drug. Abnormal laboratory findings without clinical significance (based on the PI's judgment) should not be recorded as AEs. But laboratory value changes that require therapy or adjustment in prior therapy are considered adverse events.

An adverse drug reaction is any adverse event caused by a drug. A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

9.11.2 Serious Adverse Event (SAE)

Definition: any adverse event that results in any of the following outcomes:

- Death or life-threatening event

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

Important adverse events that may not result in death, may not be life-threatening, or do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may acutely jeopardize the patient without immediate medical intervention to prevent one of the outcomes listed above. Serious may also include any other event that the investigator or company judges to be serious. The PI is responsible for reporting serious adverse events to their local IRB according to their institutional requirements. These SAEs will be captured in the CRFs as described for regular AEs.

9.11.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

9.11.4 Unanticipated Problems

Definition:

- Unexpected event (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research ("possibly related" mean 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 subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Some SAEs may meet the definition of an unanticipated problem.

9.11.5 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subject's research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

Events meeting the definition of noncompliance should be reported to the IRB in accordance with local policy.

9.11.6 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants. The IRB will determine if noncompliance is serious and/or continuing.

9.11.7 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Local IRB pre-approval of all protocol exceptions must be obtained prior to the event.

9.12 Grading of an Adverse Event

The descriptions and grading scales found in the revised NCI Common Toxicity Criteria (CTC) version 4.0 will be utilized for assessing severity of adverse events. If the toxicity is not characterized adequately by the NCI toxicity scale, the investigator will use the adjectives MILD, MODERATE, SEVERE, LIFE-THREATENING or DEATH to describe the maximum intensity of the adverse event. For purposes of consistency, these intensity grades are defined as follows:

Table 5: Grading of Adverse Events

MILD	Grade 1	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
MODERATE	Grade 2	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.
SEVERE	Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
LIFE-THREATENING	Grade 4	Urgent indication needed.
DEATH	Grade 5	Death related to AE.

9.13 Determination of Causality of Adverse Events

The relationship of the adverse event to the study treatment must be specified using the following definitions:

Table 6: Relationship of Adverse Event to the Study Treatment

None:	The event is clearly related to an event that may be due to environmental or accidental occurrence or other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.
Unlikely	The event is most likely produced by other factors such as the subject's clinical condition, therapeutic interventions, or concomitant drugs administered to the subject, **and** does not follow a known response pattern to the study drug or device.
Possible	The event follows a reasonable temporal sequence from the time of drug administration or use of device, **and/or** follows a known response pattern to the study drug or device, **but** could have been produced by other factors such as the subject's clinical condition, therapeutic interventions, or concomitant drugs administered to the subject.
Probable	The event follows a reasonable temporal sequence from the time of drug administration or use of device, **and** follows a known response pattern to the study drug or device, **and** cannot be reasonably explained by other factors such as the subject's clinical condition, therapeutic interventions, or concomitant drugs administered to the subject.
Definite	The event follows a reasonable temporal sequence from the time of drug administration or use of device, **and** follows a known response pattern to the study drug or device, **and** cannot be reasonably explained by other factors such as the subject's clinical condition, therapeutic interventions, or concomitant drugs administered to the subject, **and either** occurs immediately following study drug administration or use of device **or** improves on stopping the study drug or device, **or** reappears on repeat exposure.

9.14 Routine Adverse Event Reporting

All adverse events (as required by this protocol) must be reported in the source documentation and CRFs with appropriate information, including severity and rating of causality to the study drug/treatment. Adequate source documentation must be available to characterize the severity, duration and causality of each reported adverse event. For purposes of this protocol, The adverse event-reporting period will begin immediately following initiation of treatment, with the adjuvant treatment with TMZ and the Optune device, and end at least 30-days following last study treatment (Pembrolizumab dose, Temozolomide dose, or wearing Optune, whichever comes last). Any SAEs will be followed until stabilization.

9.15 Unanticipated Adverse Device Effect Event (UADE) Reporting

Any potential unanticipated adverse device effect (UADE) will be reported to the local IRB and DISC per local reporting policy and FDA, as applicable.

9.16 Eliciting Adverse Event Information

The investigator is to report all directly observed adverse events and all adverse events spontaneously reported by the trial patient using concise medical terminology. In addition, each trial patient will be questioned about adverse events at each clinic visit following initiation of treatment.

9.17 Adverse Event Reporting Period

The adverse event-reporting period will begin immediately following initiation of treatment, with the adjuvant treatment with TMZ and the Optune device, and end at least 30-days following last study treatment (Pembrolizumab dose, Temozolomide dose, or wearing Optune, whichever comes last). All adverse events that occur in trial patients during the adverse event reporting period must be reported on the CRFs, whether or not the event is considered study treatment-related. In addition, any known untoward event that occurs beyond the adverse event-reporting period that the investigator assesses as possibly related to the investigational medication/product should also be reported as an adverse event.

9.18 Follow-up of Unresolved Adverse Events

Adverse events will be tracked for 30 days following the last study treatment (Pembrolizumab dose, Temozolomide dose, or wearing Optune, whichever comes last). Events continuing at that time will be notes as such.

9.19 Adverse Event Reporting

All serious and unexpected adverse drug reactions must be reported to Merck Global Safety (if attributable to pembrolizumab) and FDA (as applicable). Serious adverse events will be reported to the IRB and DISC per local policy. Serious, unexpected and related SAEs will be tracked for 90 days after last following last study treatment (Pembrolizumab dose, Temozolomide dose, or wearing Optune, whichever comes last).

10. STATISTICAL CONSIDERATIONS:

10.1 Primary Endpoint

Our primary endpoint is progression-free survival time from enrollment to progression or death or censoring, whichever occurs first, with the goal of determining whether the triple combination treatment increases PFS in newly diagnosed GBM patients when compared to TTFields+TMZ historical control patients from the EF-14 study. We will use one-sample log-rank test to compare PFS between the triple combination arm relative to the historical control arm.

10.2 Secondary endpoints

We are proposing 3 secondary endpoints:

1. Toxicity and tolerability of the triple combination treatment in newly diagnosed GBM patients.
2. Overall survival time from enrollment to death or censoring, whichever occurs first() and radiographic response (RR) at 6, 12, 18 and 24 months of the triple combination in newly

diagnosed GBM, as compared to the historical control arm.

3. Augmentation of TTFields-initiated glioma-specific immune reaction by pembrolizumab.

We will estimate proportions and 95% confidence intervals for patients in the triple combination arm who experience toxicities and other types of AEs and SAEs. We will use the log-rank test to compare OS between the triple combination arm relative to the historical control arm. We will use one-sample proportion test to compare RR between the triple combination arm and historical control arm. We will use mixed effect regression to assess changes in response variables related to glioma-specific immune reaction before, during, and after treatment with pembrolizumab.

10.3 Sample Size Considerations

To determine if the proposed sample size of 24 patients enrolled in the triple combination arm will allow us to detect a clinically relevant improvement in median PFS relative to the median PFS of 6.7 months observed in the historical control arm, we assumed an accrual period of 12 months, an accrual rate of 2 patients per month, an additional 18 months of follow-up after the end of accrual, and proportional hazards. After confirming that the Weibull distribution could reasonably be used to characterize PFS times in the historical control arm, we estimated the Weibull shape parameter k from the historical control PFS curve. The shape parameter k determines if the hazard rate underlying a survival curve is accelerating over time ($k>1$), constant over time ($k=1$; identical to exponential survival), or decelerating over time ($k<1$). Based on a shape parameter estimate of $k=0.88$ with empirical 95% confidence interval (0.82,0.95) obtained via simulation of the historical control data, our proposed sample size of 24 patients in the triple combination arm should allow us to detect in improvement in PFS of 6 to 8 months with 80% power and a 1-sided significance level of 0.05 (detectable effect size methodology from Wu and Xiong, 2014 [69]).

10.4 Data Analysis Considerations (Protocol v10.1)

Dr. David Tran, sub-Investigator at USC, will assist with secondary analysis of coded clinical data for primary and secondary endpoints. A coded limited data set will be shared for purposes of data analysis and a data use agreement (DUA) will be included as part of the statement of work. Data will be stored in UF Box and Dr. Tran and his designee will be provided access via UF institutional procedures. The LDS will be transferred to USC and will be stored on secure, duallyauthenticated USC servers with limited access only to Dr. Tran and his designees. Analyzed data files will also be password protected and stored on password protected computers.

Additionally, Dr. Tran will perform correlative studies which include expression analysis of peripheral blood mononuclear cells (PBMCs) and tumor samples to determine if correlations in expression of key gene pathways with the clinical outcome of enrolled subjects with newly diagnosed GBM receiving the study treatment of adjuvant temozolomide + TTFields + pembrolizumab. PBMCs and tumor samples will include the subject's study ID, sample type and timepoint and date of collection. A corresponding coded limited data set that includes subject ID, dates associated with the individual, clinical data/survival data, treatment history and detailed response data will be shared with Dr. Tran in order to accurately identify and characterize signatures of response. We will treat survival in the 2THETOP data as a continuous variable and

develop computational models that incorporate all key clinical and molecular characteristics to define response signatures that are also continuous with correlation with survival. We plan to apply novel computational bioinformatics to identify cytotoxic T lymphocyte (CTL) clones that are shared between the tumor microenvironment (TME) and the periphery prior to treatment and at the time of radiographic changes and between pathologically proven immune changes and true recurrence. The goal is to identify T cell clones that were expanded after treatment and capable of infiltrating the TME to induce productive anti-tumor immunity. This information will help determine if immune resistance is a function of changing of the "guards" or changing in the TME's immunosuppressive functions. Each TCR clone will be paired with its expression profile to allow for the identification of the master regulatory subnetwork specific in these TME access-enabled tumor-specific CTL clones as compared to those that were expanded in the periphery but failed to reach the TME. Next, we plan to answer the following questions: 1) What defines the systemic signal created by study treatment? 2) What systemic signals distinguish objective responders from non-responders? and 3) What systemic signals may prevent non-responders from responding? For example, in certain non-responders, is it because their immune system also activates an alternate immune checkpoint (*e.g.,* LAG3, CTLA4, IDO1, TGIT etc.) in addition to PD-1/PD-L1? and if so, will using a combination of checkpoint inhibitors be more effective? The answers to these questions will help us develop a more robust signature that can predict response and guide subsequent TTFields-based combination therapy. Clinical data will be stored in UF Box and Dr. Tran and his designee will be provided access via UF institutional procedures. Sequencing data will be raw RNAseq and DNA sequences in FASTQ format. The raw sequencing data from all correlative studies for 2THETOP are stored in UF HiPerGator. UF will initiate the transfer to USC using Globus (which is a highly secure and fast transfer system frequently used in high data content transfer). Sequencing and associated clinical data will be stored on secure dually-authenticated USC supercomputer and servers with limited access to only Dr. Tran and his designees. Analyzed data files will also be password protected and stored on password protected computers.

11. STUDY CALENDAR

	Screening	Week 0 (TMZ/ Optune Cycle 1)	Week 4 (TMZ/ Optune Cycle 2, Pembro Dose 1)	Week 7 (Pembro Dose 2)	Week 8 & every 4 weeks on-treatment management (TMZ Cycles 3-12)	Week 10 & every 3 weeks on-treatment management (Pembro Dose 3 and beyond)	Post TMZ Treatment follow-up	Post-Pembro Treatment follow-up	Post treatment termination
Informed Consent	X[1]								
Medical History	X[12]								
Pregnancy Test	X[17]								
PBMC & Urine Specimen	X[18]			X[18]				X[19]	
Physical Exam, KPS	X[8]		X[8]	X[8]		X[8]	X[8]	X[8]	
Tumor Tissue	X[21]							X[22]	
PT/PTT, INR		X[23]	X[23]		X[23]		X[23]		
CBC with Differential	X	X[9]	X[9]		X[9]		X[15]	X	
CMP	X	X[10]	X[10]	X[10]	X[10]	X[10]	X[15]	X	
TSH/Free T4	X		X[11]	X[11]		X[11]	X[15]	X	
MRI	X[2]				X[13]		X[13, 20]		
Temozolomide Cycle		X[3]	X[4]		X[4]				
Pembrolizumab			X[5]	X[5]		X[5]	X[5]		
Optune		X[6]	X[6]		X[6]				
Optune Compliance			X[6]					X	
AE Assessment	X[7]	X[16]	X[16]		X[16]			X	
Concomitant Medications	X	X	X	X	X	X		X	
Survival Contact									X[14]

1. No study-specific procedures are to be performed prior to obtaining informed consent. However, assessments performed according to SOC prior to consent may be used to fulfill the screening requirement. Study treatment should start no later than 6 weeks from last dose of concomitant Temozolomide or radiation therapy (the latter of the two).
2. Patients will undergo SOC brain MRI scans with RANO disease/response evaluation at screening, 4 weeks (+7/-14 days) after end of chemoradiation.
3. Patients will begin treatment with temozolomide at 4 weeks (+14 days) from end of radiotherapy, week 0. Cycle 1 will be dosed at 150mg/m^2 daily for 5 consecutive days of a 28-day cycle.
4. Patients will begin treatment with temozolomide Cycle 2 and subsequent cycles every 4 weeks (+7 days) at 200mg/m^2 PO daily for 5 consecutive days, unless toxicity occurs. See 6.3.1 for dose escalation parameters.
5. Pembrolizumab will be given every 3 weeks (+/- 4 days) beginning no sooner than 3 weeks from the start of Optune. For subjects who receive pembrolizumab alone after the completion of TMZ or due to TMZ intolerance, pembrolizumab infusions may be administered per the treatment plan if absolute neutrophil count (ANC) is $\geq 1.0 \times 10^9$/L, and the platelet count is $\geq 50 \times 10^9$/L, and if it is determined by the treating physician that the cytopenia was unlikely to be related to pembrolizumab treatment.
6. Optune treatment will begin at Week 0 (+7 days) and will be continuous throughout study. Compliance documentation will be obtained every 4 weeks (+/- 7 days).
7. The adverse event-reporting period will begin immediately following initiation of treatment with the adjuvant treatment with TMZ and the Optune device.
8. Physical exam and KPS will be performed prior to the first and second dose of pembrolizumab, and every 3 weeks (+/- 4 days) to coincide with each subsequent pembrolizumab dose while on TMZ adjuvant therapy, then every three pembrolizumab dose thereafter. Neurological status will be included as part of the assessment.
9. CBC with Differential will be performed within 3 days prior to starting each cycle of temozolomide.
10. CMP will be performed prior to each cycle of pembrolizumab and prior to starting each cycle of temozolomide (+/- 7 days). When temozolomide and pembrolizumab are given within 3 days of each other, only one set of CMP will be done for both drugs.
11. TSH/Free T4 will be performed prior to the first infusion of pembrolizumab and then prior to every other pembrolizumab infusion thereafter.
12. Medical history may be performed at any time prior to registration.
13. Patients will undergo SOC brain MRI scans with iRANO response evaluation every 2 months (+/- 7 days) until second progression.
14. All patients will have their survival status documented every 3 months (+/- 1 month) for up to 5 years or death, whichever occurs first. Survival information can be obtained via telephone or from clinic notes.

15. If study treatment is ended due to disease progression or toxicity prior to the 24 months study term, a final research blood draw will be obtained within 30 (+/- 7) days of progression and no further research blood draws will be performed.
16. The AE reporting period will be continuous throughout the study, ending at least 30-days following last study treatment (Pembrolizumab dose, Temozolomide dose, or wearing Optune, whichever comes last).
17. Either serum or urine testing accepted.
18. Research blood draws and urine samples will be obtained prior to start of study intervention, prior to first and second dose of pembrolizumab (-14 days), 2-, 4-, 6-, 12-, and 24-months after the first dose of pembrolizumab (-7 days each dose). See section 7.1.1.
19. A final peripheral blood mono-nuclear cells will be drawn and urine specimen will be collected after end of study treatment see Section 7.1.1
20. In the case of clinical progression, an MRI will be obtained within 7 days of the treating physician becoming aware.
21. Verification of tissue availability and location needs to be determined during screening so that it can be retrieved at a later time.
22. Collection of recurrent tissue and research blood if the patient has a resection or biopsy.
23. For patients receiving relevant anti-coagulants only

12. DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the following schedule:

Table 7: Data Submission Schedule

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Registration Form Eligibility Form On-Study Form	Prior to starting treatment
Treatment Summary Form for TTFields	Every 4 weeks until completion of treatment
Treatment Summary Form for Pembrolizumab	Every 3 weeks while patient is on cycle
Treatment Summary Form for Temozolomide	Every 4 weeks while patient is on cycle
MRI Form	Baseline and every 2 months thereafter until completion of treatment
KPS Form Physical Exam Form Lab result	Per Study Calendar
Adverse Events Form	As per Section 9.0
End of Treatment Form	30 days after completion of treatment.
Survival Information	Every 3 months after completion of study therapy.

13. RESPONSE EVALUATION

For the purposes of this study, patients should be re-evaluated for response every 2 months +/- 7 Days. In addition to a baseline scan, confirmatory scans should also be obtained 2 months +/- 7 days following initial documentation of objective response. If a patient develops symptoms that are potentially indicative of disease progression, scans may be done sooner than every 8 weeks as per standard practice discretion.

Response and progression will be evaluated in this study using the updated response assessment criteria for high-grade gliomas: Response Assessment in Neuro-Oncology (RANO) [65] prior to pembrolizumab and Immunotherapy RANO (iRANO) working group guidelines once pembrolizumab has started [64] with minor modifications.

The goal of treatment compliance for this study is wearing the system for an average of no less than 75% of the time over a 4-week period for as many months as possible. Compliance reports with Optune will be obtained by Novocure's DSS per usual procedures. If a patient's compliance is below 50% over the first and second 4-week periods consecutively from the start of treatment, the patient will be trained on the importance of compliance and any barriers to compliance will be addressed. If a patient's compliance is still below 50% in the third 4-week period, the patient will be removed from study and that subject should be considered non-evaluable for survival analysis

and should be replaced. This subject however should remain evaluable for the toxicity and tolerability endpoints as per end of treatment requirement detailed in section 6.6.

13.1 Criteria for Determining First Progression Depending on Time from Initial Chemoradiotherapy

Table 8: RANO and iRANO 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 measurable contrast-enhancing lesion on decreasing, stable, or increasing doses of corticosteroids. 2. Increase by ≥ 25% in the sum of the products of perpendicular diameters between the first postradiotherapy 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 nonenhancing 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).

Note: RANO guidelines will be applied prior to first pembrolizumab infusion. After pembrolizumab treatment has been initiated, iRANO will be applied as described in Sections 13.4 and 13.5

13.2 Disease Parameters

Measurable disease: Bi-dimensionally measurable lesions with clearly defined margins by MRI scan of ≥ 10 mm. 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-measurable 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.

13.3 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 and prior to registration. An MRI should be collected and evaluated after any surgical procedure (surgical resection/debulking or biopsy).

Clinical lesions: Clinical lesions will only be considered measurable on brain MRI when they are ≥ 10 mm in bi-dimensional diameters 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 measurable 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 or amino-acid PET coupled with head CT or brain MRI: This advanced metabolic imaging technique can be used as an adjunct test to determine response or disease status. However, it should be used as the primary or sole method of determining response or disease status.

13.4 Criteria for Immunotherapy Response Assessment in Neuro-Oncology Incorporating MRI and Clinical Factors (Adapted from [64]) with modifications

The iRANO criteria integrated into the existing RANO criteria for malignant gliomas by providing recommendations for the interpretation of progressive imaging changes. Specifically, iRANO recommends confirmation of disease progression on follow-up imaging 2-3 months after initial radiographic progression if there is no new or substantially worsened neurological deficits that are not due to comorbid events or concurrent medications, and it is 6 months or less from starting immunotherapy. If follow-up imaging confirms disease progression, the date of actual progression should be backdated to the date of initial radiographic progression. The appearance of new lesions 6 months or less from the initiation of immunotherapy alone does not define progressive disease. In cases of equivocal findings, biopsy for definitive diagnosis per clinical judgment is allowed in this protocol.

For progressive imaging changes that occur more than 6 months from starting immunotherapy and if there is no new or substantially worsened neurological deficits that are not due to comorbid events or concurrent medications, and adjunct imaging tests such as perfusion/CBV and/or brain FDG-PET and/or amino-acid PET suggest a predominant treatment-induced effect, study treatment may continue and confirmation of disease progression status should be performed on follow-up imaging 2-3 months after initial radiographic progression. If follow-up imaging confirms disease progression, the date of actual progression should be backdated to the date of initial radiographic progression.

In the event of surgical intervention for clinical reasons of a radiographically suspicious lesionand then subsequent surgical pathology demonstrates treatment related changes with no obvious progression of tumor, the subject will be deemed not progressed and allowed to resume study treatment as assigned prior to the surgery at a time deemed safe by the treating physician, but no later than 8 weeks from the surgery.

13.4.1 Complete Response

Disappearance of all enhancing disease for ≥ 4 weeks; no new lesions; stable or improved T2/FLAIR; no more than physiological steroids; clinically stable or improved.

13.4.2 Partial Response

Decrease of ≥50% compared with baseline in the sum of products of perpendicular diameters of all target lesions that are sustained for at least 4 weeks; no new measurable lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved.

13.4.3 Stable Disease

Does not quality for complete response, partial response, or progressive disease; no new lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved.

13.4.4 Progressive Disease

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.; or new measurable lesions; or substantial worsened T2/FLAIR; or substantial clinical decline that is a direct cause of the tumor and cannot be attributable to a non-tumor cause.

13.5 Modified iRANO Criteria

Table 9: Key Considerations for modified iRANO

a. A repeat scan is needed to confirm radiographic progressive disease for patients without significant decline if ≤ 6 months after the start of immunotherapy.
b. The minimum time interval for confirmation of disease progression for patients without significant decline is ≥ 2-3 months per iRANO.
c. Further immunotherapy treatment is allowed ≤ 6 months after start of immune therapy after initial radiographic progressive disease (if clinically stable) pending disease progression confirmation.
d. A new measurable lesion defines progressive disease if occurring > 6 months after the start of immunotherapy, unless adjunct imaging is consistent with predominant treatment effects and there are no worsened neurological deficits that are not due to comorbid events or concurrent medications.

13.6 Response Evaluation after a Complete Resection

A complete resection will be defined as contiguous contrast enhancement < 1cm in 2 perpendicular measurements in each of 2 perpendicular planes (i.e., axial and coronal). Because neither the RECIST [70] nor MacDonald [71] criteria are completely appropriate for patients with minimal residual disease, a modified version of these criteria will be used as outlined below.

13.6.1 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.

13.6.2 Partial Response

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

13.6.3 Stable Disease

Insufficient change to qualify for Complete Response or Progressive disease.

13.6.4 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).

13.6.5 Not Assessable

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

14. QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Data and Safety Monitoring Plan

14.1.1 Data Monitoring Plan

Adverse events meeting the definition of an SAE and any grade 2 or greater AEs where there is a possible, probable or definite relationship between the AE and investigational drug must be reported to the Investigator within 24 hours of discovery. These will be assessed and reported to the IRB, DISC and FDA as required by policy and/or regulation.

The UFHCC SRMC conducts initial review of IITs and determines the level of risk which corresponds with DISC review requirements. The UFHCC DISC will conduct monitoring and audit activities as outlined in the DISC Charter. The results of such activities will be provided to the PI and IRB.

The UF study team will conduct annual QA self-assessments that include review of regulatory documentation (e.g., IRB documentation, FDA IND records, IND Sponsor-Investigator responsibilities), recruitment procedures, informed consent process/form documentation, subject selection, AE and SAE reporting, CRF and source documents.

14.1.2 Data Safety Monitoring Board

Oversight and monitoring of study related activities such as the safety of research participants, appropriateness of the study, and integrity of the data, will be provided by the University of Florida Health Cancer Center DSMB, now called the Data Integrity and Safety Committee (UFHCC DISC). DSMB reports will be submitted to the IRB per local policy.

14.2 Audits

An IRB or other local oversight group may conduct audits to evaluate compliance with the protocol and the principles of GCP. The PI will provide the auditor(s) with direct access to all relevant documents and to allocate his/her time and the time of the study team to the auditor(s) in order to discuss findings and any relevant issues.

Audits are designed to protect the rights and well-being of human research subjects. Audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon adverse events, type of study, or vulnerable populations), Phase I studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

Audits examine research studies/clinical trials methodology, processes and systems to assess whether the research is conducted according to the protocol approved by the IRB. The primary purpose of the audit/review is to verify that the standards for safety of human subjects in clinical trials and the quality of data produced by the clinical trial research are met. The audit/review will serve as a quality assurance measure, internal to the institution. Additional goals of such audits are to detect both random and systemic errors occurring during the conduct of clinical research and to emphasize "best practices" in the research/clinical trials environment.

14.3 Data Management and Processing

14.3.1 Study Documentation

Study documentation includes but is not limited to source documents, case report forms, monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the "Regulatory Binder," which includes but is not limited to signed protocol and amendments, approved and signed informed consent forms, FDA Form 1572, CAP and CLIA laboratory certifications, and clinical supplies receipts and distribution records.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include but are not limited to hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

14.3.2 Case Report Forms

Only the PI and key study personnel are permitted to make entries, changes, or corrections in the case report forms.

14.3.3 Data Management and Procedures and Data Verifications

Users of the electronic CRF will have access based on their delegated specific roles on the study.

Completeness of entered data will be checked and cross-referenced to verify accuracy. Missing or implausible data will be highlighted for the PI requiring appropriate responses (i.e. confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

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16. APPENDIX A: Karnofsky Performance Scale

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal Activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

17. APPENDIX B: RANO/iRANO Measurement Form

RANO DISEASE ASSESSMENT FORM

Study ID: **2-The-Top**						
Name:		DOB:			Subject ID:	
Baseline Scan Date:						
TARGET Lesion(s)*	**Lesion Location/ Identifier**	**Series No.**	**Image No.**	**Measurement 1 (cm)**	**Measurement 2 (cm)**	**Product (cm2)**
T1						0.00
T2						**0.00**
T3						**0.00**
T4						**0.00**
T5						0.00
					Sum of Products of Diameters (SPD)	0.00
*Measurable lesions must be contrast enhancing, have 2 perpendicular diameters >10mm & no cavity or cyst in measurement. Select a minimum of 2 & a maximum of 5 lesions. Largest lesions that are suitable for reproducible measurements are preferred.						
NON-TARGET Lesion(s)	**Lesion Location/ Identifier**	**Series No.**	**Image No.**	**Measurement 1 (cm)**	**Measurement 2 (cm)**	**Enhancing or Non-enhancing?**
NT1						
NT2						
NT3						
NT4						
NT5						
^Non-measurable lesions include lesions that are too small (<10 x 10mm), lesions that do not enhance, and lesions with a poorly defined margin. Non-target lesions can also include measurable lesions not included in the 5 target lesions.						
Dexamethasone Dose:	_____	**mg**	**Frequency:**	BID_____		
Indication:						
Clinical status:						

Intracranial Edema Physiologic Replacement Other (Specify) _____
Clear deterioration due to tumor
Clear deterioration due to non-tumor causes
Others (Specify) _____
Stable Clinically Improved Clinically

RANO DISEASE ASSESSMENT FORM cont.

Study ID: **2-The-Top**								
Name:		DOB:		Subject ID:				
Subsequent Scan Date:								
TARGE TLesion(s)*	**Lesion Location/ Identifier**	**Series No.**	**Image No.**	**Measurement 1 (cm)**	**Measurement 2 (cm)**	**Product(cm2)**	**SPD from Baseline or Best Response**	**% Change of SPD**
T1						0.00		
T2						**0.00**		
T3						**0.00**		
T4						**0.00**		
T5						0.00		
Sum of Products of Diameters (SPD)						0.00		#DIV/0
*Measurable lesions must be contrast enhancing, have 2 perpendicular diameters >10mm & no cavity or cyst in measurement. Select a minimum of 2 & a maximum of 5 lesions. Largets lesions that are suitable for reproducible measurements are preferred.								
NON-TARGE TLesion(s)^	**Lesion Location/ Identifier**	**Series No.**	**Image No.**	**Measurement 1 (cm)**	**Measurement 2 (cm)**	**Enhancing or Non-enhancing?**		
NT1								
NT3								
NT4								
NT5								
^Non-measurable lesions include lesions that are too small (<10 x 10mm), lesions that do not enhance, and lesions with a poorly defined margin. Non-target lesions can also include measurable lesions not included in the 5 target lesions.								
Dexamethasone Dose:	_____	**mg**	**Frequency:**	BID_____				
Indication:								
Clinical status								

Intracranial EdemaPhysiologic ReplacementOther (Specify) _____Clear deterioration due to tumorClear deterioration due to non-tumor causesOthers (specify) _____Stable ClinicallyImproved Clinically

18. APPENDIX C: 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.