Phase II Study of Optune Device - TT Fields plus Nivolumab and Ipilimumab for Melanoma with Brain Metastasis

Washington University School of Medicine
Division of Oncology
660 South Euclid Avenue, Campus Box 8056
St. Louis, MO 63110

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Principal Investigator: **George Ansstas, M.D.**
Phone: (314) 508-4426
E-mail: gansstas@wustl.edu

Sub-Investigators	**Modality**
Ningying Wu, Ph.D.	Biostatistics
Mona Noroozi	Clinical Research Coordinator

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Ipilimumab (Yervoy)

Study Device: Optune

[redacted]

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Protocol Revision History

Initial Approval Version	**05/02/19**
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1.0 BACKGROUND AND RATIONALE

1.1 Melanoma

The landscape of treatment for metastatic melanoma continues to rapidly change with the continued emergence of effective immunotherapeutic agents. Response to PD-1 inhibitors in front-line metastatic melanoma has been demonstrated to be approximately 30-40% and can produce both durable remissions and prolong survival. *(1,2)* T-cell inflamed tumors show remarkable sensitivity to this therapeutic class while T-cell poor tumors are generally non-responsive. *(3)* Response to the front-line combination of the CTLA-4 inhibitor ipilimumab and the PD-1 inhibitor nivolumab is approximately 60% but with substantial toxicity. *(4,5)* Additionally, the added benefit of the combination over single-agent PD-1 inhibitors may be limited to those without high PD-L1 expression. *(6)* Adding ipilimumab to PD-1 therapy, therefore, might be capturing additional responders by influencing the peri-tumoral milieu such that the cancer becomes more responsive to immune manipulation, while avoiding the added toxicity of the combination in patients who were poised to respond to anti-PD-1 therapy alone.

In the phase II CheckMate 204 study reported in The New England Journal of Medicine, Tawbi et al found that combined nivolumab (Opdivo) and ipilimumab (Yervoy) produced a high rate of intracranial clinical benefit in patients with melanoma brain metastases. In Checkmate 204, the intracranial clinical benefit rate was 57%, including complete response in 26% and partial response in 30%. Median duration of intracranial response was not reached at time of analysis *(51)*.

As also reported in the Journal of Clinical Oncology by Kluger et al, pembrolizumab (Keytruda) showed activity in brain metastases in patients with melanoma enrolled in a phase II study. The study included 23 patients with melanoma with one or more asymptomatic untreated 5- to 20-mm brain metastasis not requiring corticosteroid treatment. Prior systemic therapy had been received by 70% of patients. Brain metastasis response occurred in 6 of the total 23 patients, although 8 patients were not evaluable for brain response. Brain and extracerebral responses were concordant, with all responses ongoing at 24 months.

1.2 Nivolumab

PD-1 inhibitors as single agents in the first-line setting have been shown to consistently result in response rates of 33-44%. *(7-9)* Both pembrolizumab and nivolumab have been shown superior to ipilimumab in the front-line setting in advanced melanoma. *(10,11)* As such, PD-1 inhibitors have become standard of care in the first-line setting, either alone or in combination with ipilimumab. Data for second-line use of any therapy after PD-1 inhibitors is lacking. While ipilimumab was initially approved based on a study of patients who received the drug after failure of prior therapy, no studies of ipilimumab following failure on a PD-1 inhibitor have been reported. *(12)* As such, there are no data that guide the appropriate management of patients who have progressed on first-line immune-

checkpoint therapy with BRAF wild-type mutations.

1.2.1 Pharmacology

Mechanism of Action: Nivolumab is human monoclonal antibody which targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Nivolumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.

1.2.2 Pharmacokinetics

Distribution: Nivolumab has linear pharmacokinetics after single and multiple dosing within the range 0.1 mg/kg to 10 mg/kg. The volume distribution (Vd) is 8L.

Elimination: Clearance is independent of dose in the range 0.1 mg/kg to 10 mg/kg. The total body clearance is 9.5 mL/hr, and the elimination half-life of is approximately 26.7 days. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights.

1.3 Ipilimumab

Mechanism of Action: Cytotoxic T-lymphocyte antigen-4 CTLA-4 is a negative regulator of T-cell activity. Ipilimumab is a full human monoclonal immunoglobin (lg) antibody that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation, including the activation and proliferation of tumor infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell function, which may contribute to a general increase in T cell responsiveness, including the anti-tumor immune response.

1.3.1 Pharmacokinetics

Absorption: No formal pharmacokinetic drug interaction studies have been conducted with ipilimumab. Ipilimumab is not expected to have pharmacokinetic drug-drug interactions, since it is not metabolized by CYP450 or other drug metabolizing enzymes.

Distribution: Ipilimumab is confined mainly to the extracellular fluid. Peak concentration (Cmax), trough concentration (Cmin), and area under the plasma concentration versus time curve (AUC) of ipilimumab increased dose proportionally within the dose range examined ipilimumab is confined mainly to the extracellular fluid. Peak concentration (Cmax), trough concentration (Cmin),

and area under the plasma concentration versus time curve (AUC) of ipilimumab increased dose proportionally within the dose range examined. Based on population pharmacokinetic analysis, the mean volume of distribution (% coefficient of variation) at steady state was 7.47 liters (10%)

Metabolism: Not applicable. Monoclonal antibodies are usually degraded into amino acids and small peptides, independently from CYP450 or other drug-metabolizing enzymes.

Elimination: Clearance increased with body weight, but no dose adjustment is required with dosing on a mg/kg basis. Upon repeated dosing every 3 weeks, the clearance (CL) of ipilimumab was found to be time-invariant, and systemic accumulation was 1.5-fold or less. The mean value (% coefficient of variation) generated through population pharmacokinetic analysis for the terminal half-life (t1/2) was 15.4 days (34%) and for CL was 16.8 mL/h (38%).

1.4 Optune

Novocure has shown that when properly tuned, very low intensity, intermediate frequency electric fields (TTFields) stunt the growth of tumor cells (*13*). 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 (*14*). 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 (*15,16*) or indirectly (*17-20*) with microtubule polymerization (e.g., Taxol).

The Optune™ system (NovoTTF-200A Therapy) is a portable battery operated device, which produces TTFields within the human body by means of surface transducer arrays (*21, 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 (OS) 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 chemo 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 was 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.

1.5 Study Rationale

EF-14 trial has shown improvement in survival results in glioblastoma patients when the Optune device - TT Fields is added as conjunctive therapy to temozolomide. The 5-year survival analysis showed HR (95% CI) of 0.63 (0.53-0.76). Safety has been approved in EF-14 trial.

TTFields have also been shown to cause tumor regression in animal models and human cancers. Treating mice with a number of injected tumor models, including CT26 colon adenocarcinoma, B16/F1 melanoma, Lewis lung carcinoma, and the highly invasive VX2 carcinoma in rabbits, all demonstrated TTFields-induced tumor regression.

Chen et al. have also applied similar intermediate frequency alternating electric fields to B16/F10 melanoma cells, showing similar effects both *in vitro* and *ex vivo*. Interestingly, they also provide evidence that CD34-positive cell numbers were reduced, indicating an effect on the tumor microvasculature in the treated tumors

This phase II study will evaluate the safety of combining TT Fields with immunotherapy in melanoma patients with brain metastasis. The data of this study will also inform whether this combination will offer advantage in progression free survival (PFS) and overall survival.

1.6 Correlative Studies Background

We plan to collect blood samples to evaluate circulating tumor DNA and correlate with imaging.

2.0 OBJECTIVES

2.1 Primary Objective

To determine the progression-free survival of treating melanoma with metastasis to the brain (intracranial and extracranial) with the combination of Optune and immunotherapy.

2.2 Secondary Objectives

1. To evaluate the safety of treating melanoma with metastasis to the brain with the combination of Optune and immunotherapy by CTCAE version 5.0.
2. To determine the overall survival of treating melanoma with metastasis to the brain with the combination of Optune and immunotherapy.

2.3 Exploratory Objective

To correlate ctDNA analysis with imaging results.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Histologically or cytologically confirmed melanoma with metastasis to the brain.

2. Measurable disease defined as lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 10 mm with CT scan or MRI, as ≥ 20 mm by chest x-ray, or ≥ 10 mm with calipers by clinical exam.

3. Candidate for treatment with immunotherapy.

4. At least 18 years of age.

5. Normal bone marrow and organ function as defined below:
   a. Absolute neutrophil count ≥ 1,500/mcl
   b. Platelets ≥ 100,000/mcl
   c. Total bilirubin ≤ 1.5 x IULN
   d. AST(SGOT)/ALT(SGPT) ≤ 3.0 x IULN
   e. Creatinine ≤ IULN OR creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal

6. Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation, including at least 5 months (for women of childbearing potential) and at least 7 months (for men) after last dose of study drug. Should a woman become pregnant or suspect she is pregnant while participating in this

study, she must inform her treating physician immediately.

7. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.2 Exclusion Criteria

1. Received treatment in the metastatic setting.

2. Treated with whole brain radiation.

3. Receiving targeted therapy or on immunosuppressive agents (dexamethasone> 4mg/day) within 1 week of therapy.

4. A history of other malignancy ≤ 5 years previous with the exception of basal cell or squamous cell carcinoma of the skin which were treated with local resection only or carcinoma *in situ* of the cervix.

5. Currently receiving any other investigational agents.

6. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to nivolumab, ipilimumab, or other agents used in the study.

7. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia.

8. History of pre-existing immunodeficiency disorder or autoimmune condition requiring immunosuppressive therapy. This includes inflammatory bowel disease, ulcerative colitis, Crohn's disease, systemic vasculitis, scleroderma, psoriasis, multiple sclerosis, hemolytic anemia, immune-mediated thrombocytopenia, rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome, sarcoidosis, or other rheumatologic disease or any other medical condition or use of medication which might make it difficult for the patient to complete the full course of treatments or to generate an immune response to vaccines.

9. Known sensitivity to conductive hydrogels.

10. Skull defects such as missing bone or bullet fragments.

11. Implanted pacemaker, programmable shunts, defibrillator, deep brain stimulator, vagus nerve stimulator, and other implanted electronic devices in the brain or spinal cord.

12. Pregnant and/or breastfeeding. Women of childbearing potential must have a negative pregnancy test within 14 days of study entry.

13. Known HIV-positivity on combination antiretroviral therapy because of the potential for pharmacokinetic interactions with nivolumab and/or ipilimumab. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.3 Inclusion of Women and Minorities

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

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Study Treatment

Participants will receive nivolumab + ipilimumab followed by nivolumab alone as per standard of care. Recommended dosing is as follows:

- Ipilimumab at 3 mg/kg IV over 90 minutes on Day 1 of each 21-day cycle for 4 cycles.
- Nivolumab at 1 mg/kg IV over 30 minutes on Day 1 of each 21-day cycle for 4 cycles, then at 240 mg IV over 30 minutes on Days 1 and 15 of each 28-day cycle.

During Cycles 1 through 4, ipilimumab should start 30 minutes after the end of nivolumab.

Changes may be made to treatment with nivolumab and ipilimumab at the discretion of the treating physician. Dosing weight is defined as screening weight.

Treatment with Optune will occur as follows:
Within 2 weeks of the start of ipilimumab (before or after), treatment with Optune will begin. Patients will be instructed on the operation of the Optune device by a representative from Novocure. This training may occur in clinic or at the patient's home or at another mutually agreeable location. Training will include battery replacement and recharging, turning the device on and off, disconnecting and reconnecting the electrodes from the device for personal needs, how to handle device error messages, how to handle irritated skin, and shaving instructions. Once the patients are trained in operating the device, they will be instructed to continue treatment at home.

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. It is recommended that treatment with the device be continuous with breaks allowed for personal needs (e.g., showering, array exchange). Breaks should be no more than 1 hour twice daily. Use compliance is aimed at a minimum of 60% (14.4 hours) with the goal of achieving at least 75% (18 hours) of the time per day on average. The device log will be downloaded periodically to assess for compliance. In addition, investigators may grant brief breaks in device treatment that last no more than 7 days in any 30 day period at the investigator's discretion.

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 5.3.

5.2 Toxicity and Response Evaluations

All patients who receive any study treatment are evaluable for toxicity. Patients are evaluated from first receiving study treatment until a 100-day follow up after the conclusion of treatment or death.

All patients are evaluable for disease response unless they discontinue treatment prior to completion of Cycle 2 and have not had any disease assessment.

Patients who discontinue treatment with nivolumab and/or ipilimumab may continue to receive treatment with Optune on study. Patients who discontinue treatment with Optune may continue to receive treatment with nivolumab and/or ipilimumab on study.

5.3 General Concomitant Medication and Supportive Care Guidelines

Premedication associated with standard drug administration and supportive care (including anti-diarrheals, antibiotics, diuretics or other medications) may be given as indicated by the current American Society of Clinical Oncology (ASCO) guidelines. Premedication for the use of prophylaxis for infusion reactions (e.g., diphenhydramine, acetaminophen, or other medications) may be given per institutional standard.

For patients that experience infusion reactions the following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen/paracetamol 325 to 1000 mg at least 30 minutes before additional nivolumab or ipilimumab administrations.

As there is potential for hepatic toxicity with nivolumab or nivolumab/ipilimumab combination, drugs with a predisposition to hepatic toxicity should be used with caution in patients treated with nivolumab containing regimen.

5.3.1 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 medical alcohol. 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.

5.3.2 Prohibited Medications

The following medications are prohibited during the study (unless utilized to treat a drug related adverse event):

- Immunosuppressive agents
- Immunosuppressive doses of systemic corticosteroids (e.g., prednisone >10 mg daily or equivalent)
- Any concurrent anti-neoplastic therapy (i.e., chemotherapy, hormonal therapy, immunotherapy, extensive, non-palliative radiation therapy, or standard or investigational agents for treatment of melanoma)

5.4 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative pregnancy test within 14 days prior to the first day of study treatment.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 5 months following the last dose of nivolumab (women) and 7 months following the last dose of nivolumab (men).

If a patient is suspected to be pregnant, all study treatment should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 5 months after the last dose of nivolumab (women) or 7 months after the last dose of nivolumab (men), the investigator must be notified in order to facilitate outcome follow-up.

5.5 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue for up to one year or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death

- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unable to receive further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.6 Duration of Follow-up

Patients will be followed up at three time points: 30 days from the patient's discontinuation date, again at 100 days after the discontinuation of study treatment for adverse events, and finally at 3 years after off-treatment date. At these time points, the medical record will be reviewed to look at progression and survival endpoints. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

Dose modifications for ipilimumab and nivolumab will be made at the discretion of the treating investigator following standard of care guidelines.

There are no dose modifications permitted for Optune. Investigators may grant brief breaks in device treatment that last no more than 7 days in any 30-day period at their discretion. Reasons for breaks in treatment longer than 24 hours will be documented.

Patients who discontinue treatment with nivolumab and/or ipilimumab may continue to receive treatment with Optune on study. Patients who discontinue treatment with Optune may continue to receive treatment with nivolumab and/or ipilimumab on study.

7.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 7.2.

Novocure requires that all events be reported as outlined in Section 7.4.

7.1 Definitions

7.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

7.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- 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

7.1.3 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).

7.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

7.1.5 Unanticipated Problems

Definition:

- unexpected (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" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects 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.

7.1.7 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.

7.1.8 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.

Pre-approval of all protocol exceptions must be obtained prior to the event.

7.2 Reporting to the Human Research Protection Office (HRPO) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.

- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

7.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a QASMC auditor.

7.4 Reporting to Novocure

SAEs related to the device should be reported via email to support@novocure.com, or by calling 1-855-281-9301 (toll free).

7.5 Timeframe for Reporting Required Events

Adverse events will be tracked for 100 days after the end of study treatment.

8.0 PHARMACEUTICAL INFORMATION

8.1 Nivolumab (Opdivo)

8.1.1 Nivolumab Description

Nivolumab is a programmed death receptor-1 (PD-1) blocking antibody indicated for the treatment of patients with:

- BRAF V600 wild-type unresectable or metastatic melanoma, as a single agent
- BRAF V600 mutation positive unresectable or metastatic melanoma, as a single agent
- Unresectable or metastatic melanoma, in combination with ipilimumab
- Metastatic non-small cell lung cancer and progression on or after platinum-based chemotherapy

- Advanced renal cell carcinoma who have received prior anti-angiogenic therapy
- Classical Hodgkin lymphoma that has relapsed or progressed after autologous hematopoietic stem cell transplantation and post-transplantation brentuximab vedotin
- Recurrent or metastatic squamous cell carcinoma of the head and neck with disease progression on or after a platinum-based therapy

It is a human monoclonal antibody that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2. Nivolumab is an IgG4 kappa immunoglobulin that has a calculated molecular mass of 146 kDa.

8.1.2 Clinical Pharmacology

Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T-cell proliferation and cytokine production. Upregulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors. Nivolumab is a human immunoglobulin G4 (IgG4) monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response. In syngeneic mouse tumor models, blocking PD-1 activity resulted in decreased tumor growth.

8.1.3 Pharmacokinetics and Drug Metabolism

The pharmacokinetics (PK) of nivolumab was studied in patients over a dose range of 0.1 to 20 mg/kg administered as a single dose or as multiple doses of nivolumab every 2 or 3 weeks. Nivolumab clearance decreases over time, with a mean maximal reduction (% coefficient of variation [CV%]) from baseline values of approximately 24.5% (47.6%) resulting in a geometric mean steady state clearance (CLss) (CV%) of 8.2 mL/h (53.9%); the decrease in CLss is not considered clinically relevant. The geometric mean volume of distribution at steady state (Vss) (CV%) is 6.8 L (27.3%), and geometric mean elimination half-life is 25 days (77.5%). Steady-state concentrations of nivolumab were reached by 12 weeks when administered at 3 mg/kg every 2 weeks, and systemic accumulation was approximately 3-fold. The exposure to nivolumab increased dose proportionally over the dose range of 0.1 to 10 mg/kg administered every 2 weeks.

8.1.4 Supplier(s)

Nivolumab will be supplied as a standard of care.

8.1.5 Dosage Form and Preparation

Nivolumab will be provided as a 100 mg/10 mL (10 mg/mL) solution in a single-

use vial.

- Withdraw the required volume of nivolumab and transfer into an intravenous container.
- Dilute nivolumab with either 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP, to prepare an infusion with a final concentration ranging from 1 mg/mL to 10 mg/mL.
- Mix diluted solution by gentle inversion. Do not shake.
- Discard partially used vials or empty vials of nivolumab.

8.1.6 Storage and Stability

The product does not contain a preservative.

After preparation, store the nivolumab infusion either:

- at room temperature for no more than 8 hours from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion or
- under refrigeration at 2°C to 8°C (36°F-46°F) for no more than 24 hours from the time of infusion preparation.

Do not freeze.

8.1.7 Administration

Administer the infusion over 30 minutes through an intravenous line containing a sterile, nonpyrogenic, low protein binding in-line filter (pore size of 0.2 micrometer to 1.2 micrometer).

Do not coadminister other drugs through the same intravenous line.

Flush the intravenous line at end of infusion.

8.2 Ipilimumab (Yervoy)

8.2.1 Ipilimumab Description

Ipilimumab is a recombinant, human monoclonal antibody that binds to the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). Ipilimumab is an IgG1 kappa immunoglobulin with an approximate molecular weight of 148 kDa. Ipilimumab is produced in mammalian (Chinese hamster ovary) cell culture.

8.2.2 Clinical Pharmacology

CTLA-4 is a negative regulator of T-cell activity. Ipilimumab is a monoclonal antibody that binds to CTLA-4 and blocks the interaction of CTLA-4 with its

ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation, including the activation and proliferation of tumor infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell function, which may contribute to a general increase in T cell responsiveness, including the anti-tumor immune response.

8.2.3 Pharmacokinetics and Drug Metabolism

The pharmacokinetics (PK) of ipilimumab was studied in 785 patients with unresectable or metastatic melanoma who received doses of 0.3, 3, or 10 mg/kg once every 3 weeks for 4 doses. The PK of ipilimumab is linear in the dose range of 0.3 to 10 mg/kg. Following administration of ipilimumab every 3 weeks, the systemic accumulation was 1.5-fold or less. Steady-state concentrations of ipilimumab were reached by the third dose; the mean Cmin at steady state was 19.4 mcg/mL at 3 mg/kg and 58.1 mcg/mL at 10 mg/kg every 3 weeks. The mean value (percent coefficient of variation) based on population PK analysis for the terminal half-life (t1/2) was 15.4 days (34%) and for clearance (CL) was 16.8 mL/h (38%).

The effect of renal impairment on the CL of ipilimumab was evaluated in patients with mild (GFR <90 and ≥60 mL/min/1.73 m^2; n=349), moderate (GFR <60 and ≥30 mL/min/1.73 m^2; n=82), or severe (GFR <30 and ≥15 mL/min/1.73 m^2; n=4) renal impairment compared to patients with normal renal function (GFR ≥90 mL/min/1.73 m^2; n=350) in population PK analyses. No clinically important differences in the CL of ipilimumab were found between patients with renal impairment and patients with normal renal function

8.2.4 Supplier

Ipilimumab is supplied as a standard of care.

8.2.5 Dosage Form and Preparation

Ipilimumab (Yervoy) will be provided as a 200 mg/4 mL (50 mg/mL) solution in a single-use vial.

- Do not shake product.
- Inspect parenteral drug products visually for particulate matter and discoloration prior to administration. Discard vial if solution is cloudy, there is pronounced discoloration (solution may have pale-yellow color), or there is foreign particulate matter other than translucent-to-white, amorphous particles.

<u>Preparation of Solution</u>

- Allow the vials to stand at room temperature for approximately 5 minutes prior to preparation of infusion.
- Withdraw the required volume of ipilimumab and transfer into an intravenous bag.

- Dilute with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare a diluted solution with a final concentration ranging from 1 mg/mL to 2 mg/mL. Mix diluted solution by gentle inversion.
- Store the diluted solution for no more than 24 hours under refrigeration (2°C to 8°C, 36°F to 46°F) or at room temperature (20°C to 25°C, 68°F to 77°F).
- Discard partially used vials or empty vials of ipilimumab.

Administration Instructions

- Do not mix ipilimumab with, or administer as an infusion with, other medicinal products.
- Flush the intravenous line with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP after each dose.
- Administer diluted solution over 90 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter.

8.2.6 Storage and Stability

Store ipilimumab under refrigeration at 2°C to 8°C (36°F to 46°F). Do not freeze. Protect vials from light.

8.2.7 Administration

Please refer to Section 5.2.

8.2.8 Special Handling Instructions

None.

9.0 DEVICE INFORMATION

9.1 Optune

9.1.1 Description

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 (*23*). 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 (*23,24*). In addition, low frequency pulsed electric fields have been claimed to stimulate bone growth and accelerate fracture healing (*25*). 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 (i.e., above many 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 (*26*). 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 (*27*). 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 (*26*). 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) and cell rotation (*28-30*). 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 (*31, 32*).

9.1.2 Supplier

Novocure has shown that when properly tuned, very low intensity, intermediate frequency electric fields (TTFields) stunt the growth of tumor cells (*33*). 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 (*34*). 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 (*35, 36*) or indirectly (*37-40*) with microtubule polymerization (e.g., Taxol).

Optune is a portable battery operated device, which produces TTFields within the human body by means of surface transducer arrays (*41, 42*). 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 (OS) of patients treated with the device was equivalent to those treated with standard chemotherapy alone (*42*). Six-month progression-free survival (6PFS) analysis favored the TTFields arm compared to the chemo 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 was also no increased grade 3 and 4 central nervous system adverse events. The most common device specific adverse event was skin rash due to the transducer arrays. In EF 14 trial, 695 glioblastoma patients randomized 2:1 to TTFields plus maintenance temozolomide chemotherapy (n = 466) or temozolomide alone (n = 229). Median progression-free survival from randomization was 6.7 months in the TTFields-temozolomide group and 4.0 months in the temozolomide-alone group (HR, 0.63; 95% CI, 0.52-0.76; P < .001). Median overall survival was 20.9 months in the TTFields-temozolomide group vs 16.0 months in the temozolomide-alone group (HR, 0.63; 95% CI, 0.53-0.76; P < .001).

9.1.3 Guidance

A post hoc subgroup analysis of the EF-11 study data revealed that patients with bevacizumab-refractory GBM achieved median OS of 6.3 months when treated with TTFields monotherapy compared to 3.3 months when standard bevacizumab-based chemotherapy was continued (HR 0.39, 95% CI 0.19-0.79, p=0.01). This preliminary data suggest that bevacizumab-refractory GBM may be particularly sensitive to the anti-mitotic activity of TTFields (*43,44*) after bevacizumab withdrawal. Rapid tumor regrowth and radiographic tumor rebound phenomenon has been reported after withdrawal of bevacizumab in patients with recurrent high-grade glioma, leading to an accelerated clinical decline (*45*). For this reason, many practitioners are reluctant to discontinue bevacizumab even when bevacizumab failure is well documented, and thus this approach represents a potential overtreatment. Molecularly, acquired resistance to bevacizumab in high grade gliomas is thought to result from a meschymal transition characterized by an enrichment of slow-cycling, highly invasive, and treatment-resistant glioma stem-

like cells (GSCs) (*46, 47*). After discontinuation of bevacizumab, the microhypoxic stress and pro-GSC environment are removed and presumably GSCs exit cell cycle arrest and reactivate rapid proliferation. This is during bevacizumab withdrawal that we hypothesis that GBM cells, especially GSCs reentering rapid cycling, are most sensitive to the anti-mitotic activities of TTFields. Bevacizumab treatment may be resumed temporarily for subsequent radiographic progression or worsening symptoms, due to either further disease progression or inflammatory responses to TTFields-induced cell death. Once inflammation is controlled, bevacizumab is again withdrawn and this cycle can be repeated many times. We hypothesize that successive cycles of on/off (or pulsed) bevacizumab dosing will produce peaks and troughs in mitotic activities of GSCs that allows TTFields to work at slowly reducing the GSC fraction in tumors, and thus lowering disease infiltration and increasing survival.

We recently conducted a case series study describing the outcomes for 8 patients with bevacizumab-refractory GBM, who were treated with TTFields monotherapy first, then with concomitant re-challenge with bevacizumab upon subsequent radiographic progression in 5 of these patients (*48*). The 8 patients had a median OS of 216 days (7.2 months) from first day of treatment with TTFields therapy, which was significantly longer than historical data of 1-4 months and consistent with the subgroup analysis of EF-11 data. Of the 5 patients re-challenged with bevacizumab, median OS from first dose of bevacizumab re-challenge was 172 days (5.7 months), although they all had developed resistance to bevacizumab after having received a median time of 236.5 days (7.9 months) of bevacizumab prior to starting TTFields. Patients highly adherent to treatment -- who were also those who received pulse dose bevacizumab -- had the longest OS. Patient # 4 had an adherence rate of 92.9%; her time from first day of TTFields therapy to death was 276 days (9.2 months) and, from first dose of bevacizumab re-challenge until death, 150 days (5.0 months). Patient # 7, with an adherence rate of 73.2%, had a time from first day of TTFields to death of 406 days (13.5 months) and, from first dose of bevacizumab re-challenge until death, of 349 days (11.6 months) (Fig. 1).

In this case series, we describe 8 patients with recurrent glioblastoma in whom we discontinued treatment with bevacizumab successfully - despite the concern of possible rapid disease progression upon bevacizumab withdrawal, effectively used TTFields therapy, and then re-challenged them with bevacizumab once patients developed symptoms and/or had evidence of radiographic progression. This "pulsed dosing" approach to bevacizumab administration, combined with TTFields therapy, has not been described in this patient population, and although the results from this small study is encouraging this novel approach will need to be formally tested in a focused prospective clinical study.

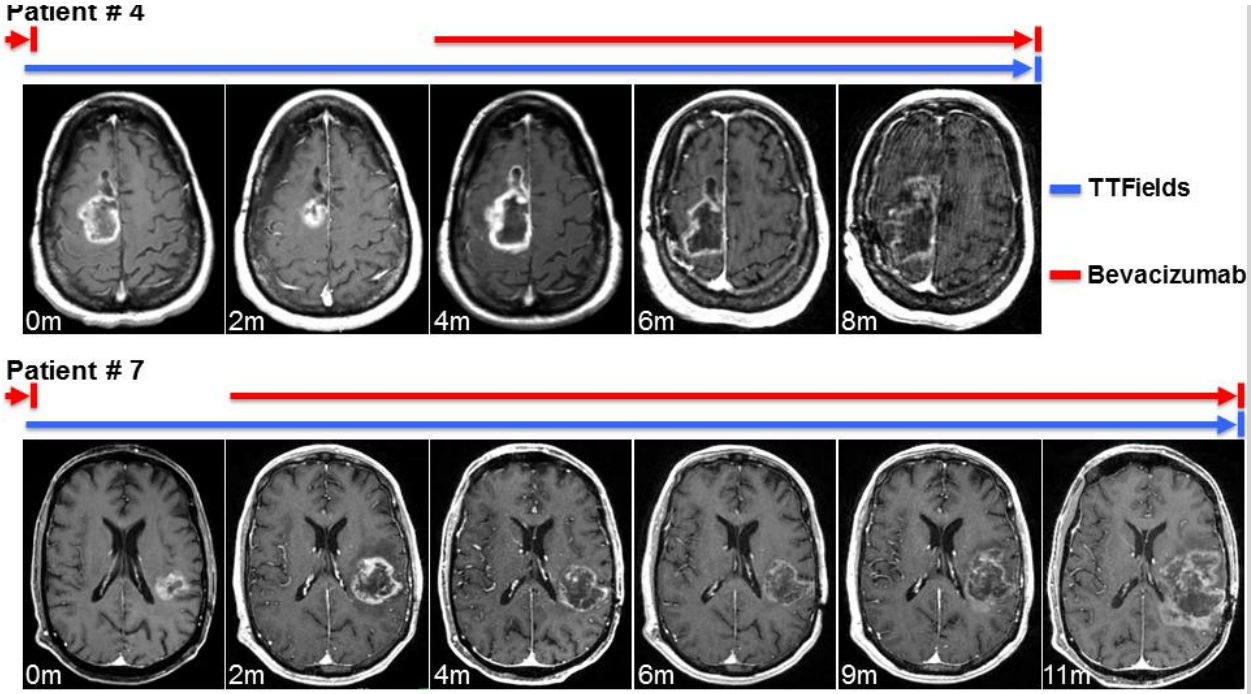

Figure 1: Radiographic appearance of bevacizumab-refractory GBM treated with TTFields and subsequently re-challenged with bevacizumab. Representative pictures of serial gadolinium contrast-enhanced brain MRI scans of Patients # 4 and # 7 are shown. Colored bars denote time line of TTFields and bevacizumab re-challenge in months, starting from the first documented radiographic diagnosis of bevacizumab-refractory GBM per RANO criteria. Patient # 4 demonstrated an initial response to TTFields at 2 months but progressed radiographically at 4 months. Upon re-challenge with bevacizumab, this patient's GBM demonstrated a radiographic response again. Patient # 7 did not have a radiographic response to TTFields in the first 2 months. However, this patient had a durable radiographic response to bevacizumab re-challenge while continuing on with TTFields.

10.0 OPTIONAL CORRELATIVE STUDIES

Twenty mL of blood will be collected at baseline, Week 3, and Week 12 in a cell-free DNA BCT (Streck) tube for patients who consent to this optional collection. Specimens will be taken to Dr. David Chen in the Department of Dermatology or will be frozen and stored.

11.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans and x-rays must be done no more than 4 weeks prior to the start of the protocol therapy.

	Screening[10]	**Baseline**[10]	**Q3W x4**[10]	**Then Q2W x6**[10]	**Then Q2W x14**[10]	**EOT**[9, 10]	**F/U**[10]
Informed consent	X						
Physical exam	X		X	X	X[8]	X	
CBC	X		Per SOC				
CMP	X		Per SOC				
TSH w/free T3 and T4			Per SOC				
Pregnancy test[1]	X						
CT C/A/P RECIST	X			Q8W for 12 mos, then Q12W for 12 mos			
Brain MRI	X						
Ipilimumab			X[2]				
Nivolumab			X[3]	X[3]	X[3]		
Optune			Daily for 75% of each day[7]				
Research blood		X	X[6]				
AE assessment		X ----------					X[4]
Follow-up							X[5]

1. Women of childbearing potential
2. Ipilimumab is being given as per SOC; recommended dosing is Q3W x4
3. Nivolumab is being given as per SOC; recommended dosing is Q3W x4, then Q2W for up to 20 doses or until progression or intolerable side effect
4. AEs are collected from baseline through 100 days after last day of treatment
5. Follow-up consists of 100-day follow-up for AEs and then review of the medical record at 3 years after last day of treatment
6. Blood for research is drawn during treatment at Week 3 and Week 12
7. Treatment with Optune will start no more than 2 weeks before or after the start of ipilimumab.
8. Q4W till off treatment.
9. May take place up to 30 days from last treatment (with immunotherapy or Optune).
10. (+/- 7 days)

12.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form	Prior to starting treatment
Treatment Form	Every cycle
Toxicity Form	Continuous
Treatment Summary Form	Completion of treatment
Research Blood Form	Baseline, Week 3, Week 12
Follow Up Form	Day 30, Day 100, Year 3
RANO Form	Baseline, every 8 weeks for 1 year, every 12 weeks for 1 year
Progression Form	Time of progression
Death Form	Time of death

13.0 MEASUREMENT OF EFFECT

13.1 RANO

13.1.1 Antitumor Effect – RANO

Brain MRI will be performed Q8W for 12 months then Q12W for 12 months.

Criteria for response: Response will be evaluated in this study using the updated response assessment criteria for high-grade gliomas: Response Assessment in Neuro-Oncology (RANO) working group guideline.

Criteria for Response Assessment Incorporating MRI and Clinical Factors

Response	Criteria
Complete response	• Requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks. • No new lesions; stable or improved nonenhancing (T2/FLAIR) lesions. • Patients must be off corticosteroids (or on physiologic replacement doses only) and stable or improved clinically. Note: Patients with nonmeasurable disease only cannot have a complete response; the best response possible is stable disease.
Partial response	Requires all of the following: • ≥ 50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. • No progression of nonmeasurable disease. • Stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.

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

- NOTE. All measurable and nonmeasurable lesions must be assessed using the same techniques as at baseline.
- Abbreviations: MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery.
- Stable doses of corticosteroids include patients not on corticosteroids.

Criteria for progression: Pseudoprogression is a phenomenon where radiographic features are consistent with tumor recurrence/progression but is instead related to treatment effect. This is a common occurrence in glioblastoma following radiation that is generally seen in the first 3-6 months after completing therapy.

If follow-up imaging confirms progression, the date of actual progression should be back-dated to the date of initial radiographic evidence of progression.

Alternatively, progressive disease can be defined as radiographic evidence of progression PLUS significant clinical decline that is felt to be unrelated to a co-morbid event or concurrent medication.

13.1.2 Disease Parameters

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

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

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

Target lesions: All measurable lesions that are residual of the lesion treated with MLA should be identified as target lesions and recorded and measured. 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.

13.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler.

Clinical lesions: Clinical lesions will only be considered measurable on brain MRI when they are ≥ 5 mm diameter as assessed using a ruler.

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

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

13.1.3.1 Evaluation of Target Lesions

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

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

for at least 4 weeks.

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

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

13.1.3.2 Evaluation of Non-Target Lesions

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

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

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy not caused by comorbid events (e.g. radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). Unequivocal progression should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

13.1.3.3 Evaluation of Best Overall Response

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

Summary of the RANO Response Criteria

Criterion	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	< 50% ↓ but < 25% ↑	≥ 25% ↑[*]
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑[*]

Criterion	CR	PR	SD	PD
New lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA†
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for response	All	All	All	Any*

Abbreviations: RANO, Response Assessment in Neuro-Oncology; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FLAIR, fluid-attenuated inversion recovery; NA, not applicable.
* Progression occurs when this criterion is present.
† Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

13.1.3.4 Duration of Response

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

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

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

13.1.3.5 Progression-Free Survival

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

13.2 RECIST

13.2.1 Antitumor Effect – RECIST

For the purposes of this study, patients should be re-evaluated for response every third cycle. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[17]. Changes in the largest diameter

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

13.2.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as >20 mm by chest x-ray, as >10 mm with CT scan, or >10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

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

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

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

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

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

13.2.3 Methods for Evaluation of Measurable Disease

All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment. CT is the preferred and standard imaging modality.

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

MRI: Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. As with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if it can be documented that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. If there is concern about radiation exposure due to CT, MRI may be used instead of CT in selected instances.

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response, stable disease or progressive disease.

13.2.4 Response Criteria

13.2.4.1 Evaluation of Target Lesions

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

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

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

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

13.2.4.2 Evaluation of Non-Target Lesions

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

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

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

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

13.2.4.3 Evaluation of Best Overall Response

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

achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	>4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	>4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once >4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

13.2.4.4 Duration of Response

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

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

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

13.2.4.5 Progression-Free Survival

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

14.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

During the phase II, the Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules

- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

15.0 STATISTICAL CONSIDERATIONS

15.1 Study Design

This is an open-label, single center, one cohort, non-randomized, phase II study. The aim is to evaluate the efficacy and safety of the combination of Optune and immunotherapy in melanoma patients with metastasis to the brain.

15.2 Primary Endpoint

Primary endpoint is intracranial progression-free survival (PFS) at 6 months. The PFS time will be calculated as the duration of time from the date of first dose of study treatment to the date of earliest intracranial progression or death, whichever occurs first. Patients who neither progress nor die by the data cutoff date will be censored at the last follow up. Intracranial response and progression will be determined by the investigator using modified RANO criteria.

15.3 Secondary Endpoints

- Safety and tolerability will be defined as the number of treatment-related grade 3 or greater adverse events (AEs) and discontinuations due to treatment-related AEs. Adverse events will be assessed using CTCAE v5.0 criteria. Safety will be monitored from the time of initiation of study treatment to 30 days after discontinuation of therapy.

- Best intracranial response rate, defined as the percentage of patients with a confirmed intracranial complete or partial response, using modified RANO criteria.

- Best extracranial response rate, defined as the percentage of patients with a confirmed extracranial complete or partial response, using modified RANO criteria.

- Extracranial progression-free survival at 6 months, defined as the duration of time from the date of first dose of study treatment to the date of earliest extracranial progression or death, whichever occurs first. Patients who neither progress nor die by the data cutoff date will be censored at the last follow up. Response and progression will be determined by the investigator using modified RECIST version 1.1 criteria.

- Overall survival (OS) at 6 months, defined as the duration of time from the date of first dose of study treatment to death from any cause. Patients who are alive by the data cutoff date will be censored at the last follow up.

15.4 Accrual

In total, 23 patients will be enrolled into the study. Non-random convenience sampling will be used. On average, we anticipate that we will enroll 1-2 patients per month. We expect to complete enrollment within 24 months.

15.5 Power Analysis

Previously published study [Long GV Lancet Oncol 2018] suggested a 53% intracranial 6-month progression-free survival rate for patients without study treatment. For a one sample clinical trial, to evaluate 6-month progression free survival, given accrual length is 2 years and follow-up length is 6 months, with one-sided significant level of 0.05, assuming the proportion of progression free under null hypothesis at 6-month is 53% and the proportion of progression free under alternative hypothesis at 6-month is 75%, a sample size of 23 could yield a power no less than 0.8.

15.6 Data Analysis

All data will be evaluated as observed, and no imputation method for missing values will be used. All data will be presented in a descriptive manner. All analyses will be considered as exploratory, even if statistical tests will be used. Descriptive statistics will be used to summarize the trial results, i.e., statistics for continuous variables may include means, medians, ranges and appropriate measures of variability. Qualitative variables will be summarized by counts and percentages. The uncertainty of estimates will be assessed by confidence intervals (CI).

PFS and OS will be presented in patient listings and analyzed via the Kaplan-Meier method. Median survival and corresponding two-sided 95% CIs will also be computed. Survival rates at 6 months with 95% CIs will be estimated using the Kaplan-Meier method. Safety data will be summarized by the number and proportions of patients affected in each cohort. Further exploratory correlative analyses will be performed via Fisher exact test, t-test, or nonparametric Wilcoxon–Mann–Whitney test, if needed.

15.7 Safety Early Stopping Rule

Safety will be reviewed on a continuous basis. Early stopping of this trial is calculated based upon the report of treatment-related grade 3 or greater adverse event (AE). A Bayesian sequential monitoring design for single-arm clinical trial (Thall, Simon, Estey, 1995) is used for the calculation of toxicity stopping boundaries, under the assumption that AE rate of 55% and or less is acceptable. For a treatment cohort with sample size of 23, modeled by a toxicity prior distribution, *Beta*(0.55, 0.45), assuming the study will stop if the estimated posterior probability of the study treatment being more toxic is greater than

0.8, the study will be halted if the treatment-related AE occurs in the 4 of the first 5 patients, or 5 of the first 7, or 6 of the first 8, or 7 of the first 10, or 8 of the first 11, or 9 of the first 13, or 10 of the first 15, or 11 of the first 16, or 12 of the first 18, or 13 of the first 20, or 14 of the first 21, or if the 15th treatment-related grade 3 or above AE is observed before the last (23rd) patient has completed the trial.

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APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.