

Title: Prognosis and therapeutic biomarkers for glioblastoma patients (for research only)

Investigators: Principal Investigator, other co-investigators

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1. Introduction/Background

The purpose of this study is to investigate if the potential biomarkers identified could be used for facilitating the diagnosis and prognosis of patients with glioblastoma (GBM).

GBM, accounting for 50% of central nerve system (CNS) tumors, is a deadly disease with no effective curable therapy. To improve the quality of patients' life and enhance the therapy response, Optune was developed and approved by the US Food and Drug Administration (FDA) for treating recurrent GBM (1-9). Now both Optune and Adjuvant TMZ plus Optune treatment are considered as a standard strategy for maintenance therapy to extend patients' life after surgery and chemotherapy (2, 6). Currently, diagnosis and prognosis of GBM rely on magnetic resonance imaging (MRI), computed tomography (CT), and biopsy (10). These diagnosis and prognosis processes for patients with GBM are expensive, difficult, and inaccurate. Biopsy of brain tissues from GBM patients is a very aggressive procedure, and it is extremely difficult to re-biopsy. By analyzing previously published data derived from microarray and next generation sequencing as well as the experimental data generated from our lab, we have identified a group of genes that have potential to be used as biomarkers for GBM prognosis.

To ascertain if the potential biomarkers could be used to facilitate the diagnosis of patients with GBM, the goal of this proposed study is to determine the expression levels of selected biomarkers in GBM tissues and the cerebrospinal fluid (CSF) from patients with GBM receiving different types of treatment against controls.

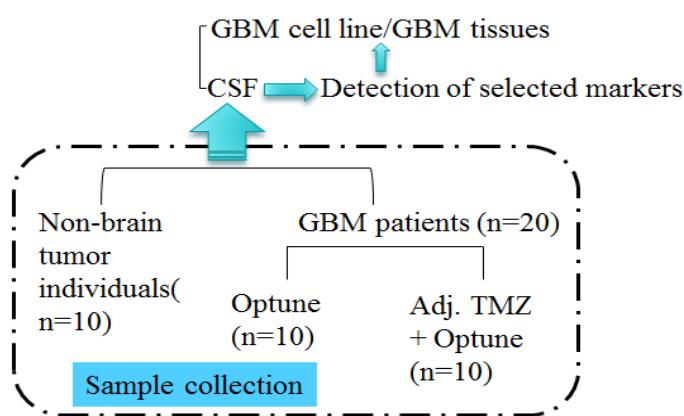
2. Significance

There is no effective therapy for GBM. There is a critical need for early diagnostic and prognostic biomarkers for GBM. The completion of this study will provide a potential biomarker for diagnosis and prognosis of GBM patients.

3. Objectives & Specific Aims

The **objective** of this proposed study is to determine the expression levels of a panel of markers including CD133, CD44, ABCC3, TNFRSF1A, AKT1, IDH2, and MGMT in GBM tissues and the CSF, blood, and saliva from patients with GBM receiving different types of treatment as described in Figure 1 and the non-brain tumor patients (controls). Our aims are: 1) To compare the differences of CD44 between the control group and the combined cancer groups at baseline; and 2) To compare the levels of CD44 and other markers among the 3 different groups such as control vs. GBM patients and Optune vs. Optune + TMZ after 8 weeks of treatment. Exploratory analysis of all other markers will be performed.

Figure 1 Overall strategy



4. Methodology

4.1 Study Design.

To reach our goal, a small clinical study will be performed with the support from NovoCure Inc. In this study, 30 participants will be enrolled in which 10 non-brain tumor patients (e.g., patients will have other types of minor neurological disorder(s) that necessitate a lumbar puncture e.g., headache, epilepsy or neurodegenerative disorders such as Alzheimer's disease or Multiple sclerosis or Parkinson's disease) will be used as the control. As shown in Figure 1, 20 patients (n=20) with GBM will be divided into two groups; patients in group 3 will receive the combination of TMZ (150-200 mg/m² orally every day; 5 consecutive days per 28-day treatment cycle for 6 cycles) with Optune and patients in group 2 will receive Optune only. (The GBM patients will be monitored for tumor size using MRI and CT scan before and after each type of treatment, which is standard of care for all GBM patients.) We will collect the CSF (5 ml), blood (2 ml), and saliva (5 ml) from each participant as indicated in Figure 2 before and after treatment (Optune alone or Optune plus TMZ for detection of biomarkers. These samples will be used for detection of selected biomarkers at gene (RNA and DNA) and protein levels. CSF is standard of care for all GBM patients, we will ask to store the leftover tumor tissues for our research.

4.2 Subject Selection/Participants.

The participants in both non-brain tumor group and GBM groups (male and/or female, there is no preference at this time) should be over 22 years old without pregnancy, or an infectious disease (cold, flu, HIV, etc.) or a blood disorder (e.g., lack of platelets, anemia, thrombosis) or vascular malformations. For controls, only patients evaluated for the neurological disorders whose evaluation required spinal taps at both the Neurology clinic and the Brain Tumor clinics will be used for this study. This is an exploratory pilot study. Based on others previous experiences, 30 subjects (10 subjects in each group) will allow us to estimate the levels of selected biomarkers in the body fluids of different groups of patients and the potential value of these selected biomarkers for GBM prognosis.

4.3 Procedures/Methods.

For the non-brain tumor control group, 5 ml of CSF, 2 ml of blood, and 5 ml of saliva will be collected one time from each participant. For the GBM patients, patients will first receive surgery to remove the tumor (the tissue will be used for diagnosis and we will use the left over for the biomarker detection) followed by radiation and/or chemotherapy according to patients' health conditions. (CSF are standard of care for these patients.) Thereafter, the physician will discuss with the patients and help patients pick their FDA approved treatment options to receive Optune or Optune plus TMZ.

All of the GBM patients will be asked for blood, saliva, and CSF withdraws at three time points as indicated in Figure 2 (the first time point is at after surgery +/- 1 week and before the combination of radiation and chemotherapy; the second time point is immediately before starting Optune/TMZ treatment +/- 3 days [at 6 weeks after the combination treatment]; and the third time point is at 8 months after the Optune/TMZ treatment +/- 1 month). The samples collected will be used to determine the levels of selected potential biomarkers at protein, DNA/RNA levels. These data will be used to assess the efficacy of the treatments for GBM patients and the correlation of the efficacies of the treatments with the expression levels of testing biomarkers in their CSF, blood, and saliva.

Method for saliva collection: Patients should be consented on day one and come back for blood and saliva withdrawal. For saliva collection, participants will be instructed to fast overnight before saliva collection. Teeth brushing should occur at least one hour prior to saliva collection. Saliva collection should (ideally) occur between 8:00 am and 10:00 am. Upon arrival at the clinic, patients should be instructed to rinse mouth with deionized (DI) water for thirty seconds (30 s) to remove any residual foreign particles (e.g., food) and/or mucus from the mouth prior to saliva collection. Water used to rinse mouth can be spit out and discarded. Wait one minute after rinsing mouth before beginning saliva collection. Saliva will be collected from both "normal patients" and "patients with the disease" using the passive drool method (i.e.,

non-chemically stimulated, whole saliva collection), as previously described. Patients should be informed that we strictly want to collect saliva, mucous and/or phlegm should not be collected. After the one minute waiting period, start a timer and instruct patients to think about eating their favorite food while making a chewing motion with their jaw (these actions help stimulate saliva production). Patients should pool saliva under their tongue and passively “drool” saliva through a drinking straw (cut to a length of approximately 2 inches (11) into a 15 mL centrifuge tube. Please keep collection tube in a cup filled with ice (crushed ice is preferable) during saliva collection to help preserve biomolecules. Final volume of saliva collected should be ~ 5 mL (no less than 3 mL). Upon completion of saliva collection, stop timer and record total time taken to collect the sample (this time will be used to estimate saliva production rates). Immediately after saliva collection, transfer 15 mL centrifuge tube to a -80 °C freezer for storage. Patients may resume a normal eating schedule immediately after completion of saliva collection.

Subject recruitment & enrollment plan

For GBM patients, there are over 60 GBM patients enrolled at Baylor Scott & White Health each year. The coordinators will screen patients' files to find potential participants of patients with GBM. The patients are at least 22 years old and without pregnancy, or an infectious disease (cold, flu, HIV, etc.) or a blood disorder (e.g., lack of platelets, anemia, thrombosis) or vascular malformations will be selected and interviewed for participating this study after discussing treatment options with their physicians.

For the participants in the control group, there are several hundreds of patients with neurological disease (e.g., patients with Alzheimer's or multiple sclerosis or Parkinson's disease) enrolled at Baylor Scott & White Health yearly. CSF and blood draws are required for diagnosis of these patients. As CSF draw is standard of care, we will be able to find those individuals from enrolled patients who have other neurological disorders such as Alzheimer's or multiple sclerosis and use the leftover CSF for our research. Thus, there is no need for advertisement. The coordinators will screen patients' files to find potential participants of patients with non-brain tumor and then the coordinators will interview those individuals and recruit them for this study. As soon as the project is approved by the IRB committee, we will start to recruit patients for this study.

Although there is little risk for patients who participant in this study, we still ask all participants to sign an Informed Consent form. All participants will be given an IRB approved Informed Consent form by their doctors before they enter in this study.

Incentives for participation/compensation

The participants will be paid for giving their blood and CSF for the tests (\$25 each time per test) after each visit. The payment will be made by from Baylor Scott & White Research Institute and it is considered taxable income. The participants must be eligible to be paid in the United States and willing to complete all the necessary tax/legal paperwork to receive this payment.

Study procedures for individual visits/interventions and/or interactions

Optune, a Novo treating tumor field (NovoTTF) device, is approved by FDA for treating patients with relapsed GBM. Recent studies indicate that it is possible for people with newly diagnosed GBM living longer when Optune is used together with the chemotherapy TMZ.

The control group will have no interaction with anything as they will not receive any anticancer treatment and all participants without brain tumor will be signed up in this group.

Data collection variables

The time points for data and sample collection will follow the schedule as indicated in Figure 2. The first point will be served as the baseline; the second point will be the intermediate; and the third point will be the endpoint for the biomarker study of using CSF samples. The data generated from the patients' samples will be kept in the lab. As these data do not contain any privacy information, there will be no privacy concern.

The data for the tumor sizes and patients' general performs will be a part of the standard care. Data collection forms can be Excel spreadsheets or created in Microsoft Access (the Data Management group can create these for the PI).

For privacy concerns, all the information about participants from this study will only be shown to the people working on the study. The results of this study may be published in a scientific book or journal. If this is done, the name of any participants will not be used. All information about participants from this research project will be kept in a locked office. Information that is kept on computers will be kept safe from access by people who should not see it. Sensitive information is not allowed to transfer or distribute to the third party without permission. All investigators who deal with collected tissue samples on this application have taken or will take the respective institutional "human subject protection" training for this project. Only Key Study Personnel (KSP) will have access to collected data and that it will be housed in secure environment. The data will not be destroyed at least for 6 year.

Tumor tissues will be collected from patients for diagnosis and future research e.g., evaluating the levels of biomarkers selected. The tissues will be snap-frozen (fresh-frozen, FF) and then stored at -80 or liquid nitrogen. Alternatively, it could be formalin-fixed paraffin-embedded (FFPE) for long time storage (10 years). All samples are expected to be used for diagnosis, thus, though the original samples may be labeled with patients' information. However, the patients' information will not be released to the researchers. The pathologist will change the label to GBM with numbers only.

The 5 ml of CSF collected from each patient each time will be centrifuged at 3500 rpm at 4°C for 10 min to remove any possible blood contamination and kept in the dry ice or stored at -80°C immediately. The samples will be used for determining the protein levels of selected potential markers. All the samples will be labeled with number system without PHI information and the samples are expected to be analyzed within 12 months. The amount of samples collected in this study is very limited and we do not expect any sample left after the study.

For the privacy concerns, only publishable data will be shared by the third party. All samples will not be transferred from Baylor Scott & White Health to a non -Scott & White Health laboratory for analysis. In case, there is a need to transfer the materials from Baylor Scott & White Health to a third party for sample analysis, permission. A Materials Transfer Agreement (MTA) or Data Use Agreement (DUA) will be required before any materials or data can be shared. Medical Research Building (MRB), an MTA is also required. The Office of Research Business Development can assist with development of these documents

Subject withdrawal

The patients have the right to leave the study anytime. The data collected will be used for the analysis as long as the data are meaningful. Otherwise, more patients will be enrolled.

Reporting adverse events or other significant events

As both Optune and TMZ are FDA approved treatment options for GBM patients, there are no significant adverse events caused by these treatment. In an unlikely case, there are any significant events; it should be reported to IRB immediately.

4.3 Power Calculation

The goal of the study is to compare the differences in the CSF, blood and saliva levels of CD44 between 1) the control group and the Optune alone and the TMZ + Optune group; and 2) Optune alone vs, TMZ + Optune alone.

There is no previous literature on CD44 levels in GBM. To calculate a sample size, we are assuming that the baseline and post treatment levels of CD44 standard form in the CSF will be approximately 327 ± 134 ng/ml and 185 ± 103 , respectively, similar to those reported by Kawano et al. for head and neck cancer(12). One of the drawbacks of the report is that they only reported the median and do not specify if it is the standard deviation or range that they reported.

For the primary goal, it is assumed that the median is similar to the mean and the reported value is the standard deviation. Further assumptions are: sample sizes of n=10 and n=20 (for the control and the cancer groups, respectively); significance level of 0.05; expected means of 327 and 185 (for the cancer and the control groups, respectively); common standard deviation of 115; independence of the sample units and normality of the observations. The power was calculated using a two sample t-test and it was found to be 87%. Power calculation was performed with PASS v.13.0.4.

4.4 Data Analysis.

Descriptive statistics will be provided. Categorical data will be reported as count (percentages). Continuous data will be reported as mean (standard deviation) or median (range) as appropriate. A two sample t-test will be used to compare the biomarker level between groups, unless the distributions are found to be non-symmetric. In that case, Wilcoxon-Rank-Sum tests will be used.

5. Human Subjects

Thirty individuals will take part in this study at Baylor Scott & White Healthcare. Body fluids we requested are considered to have concern of human subject.

Risks (including physical, emotional, social, and/or privacy)

Risks of Radiation – Diagnostic Test: Patients will have the same amount of radiation exposure regardless of whether the participant elects to take part in this research study or receives standard medical care.

Risks of participation in the study for individuals using Optune alone or Optune plus TMZ: TMZ is commonly used to treat patients with GBM. Common side effects of patients using TMZ include nausea, vomiting, loss of appetite, constipation, diarrhea, skin rash, tiredness, weakness, dizziness, blurred vision, sleep problems (insomnia), unusual or unpleasant taste in your mouth, and headache. Optune is a FDA approved device for GBM patients as well. In a large clinical study, patients who received Optune for at least 18 hours a day responded better to treatment. Because Optune works differently, it does not cause many of the side effects associated with chemotherapy. The most common side effect reported is skin irritation occurring under the transducer array. These reactions go away shortly after the TMZ and/or Optune are stopped. We are studying possible risks and reactions related to the TMZ and Optune. Current studies show that both TMZ and Optune are very safe to be used for GBM patients and no risk related to the use of TMZ and/or Optune has been reported so far.

Risks of blood and CSF withdrawals: Withdrawal of CSF (5 ml), blood (2 ml), and saliva (5 ml) from patients is a standard practice in the hospital. In general we do not expect more than a minor risk for patients. However, patients after withdrawal of CSF may have back pain for short time, or bleeding (this only happens to the patients with bleeding disorder and these subjects are excluded from the study) or infection. The incidence of having infection is very rare as the procedure will be very clean. In the case of back pain, patients will receive pain killer such as nonsteroidal anti-inflammatory drugs (NSAIDs), or Ibuprofen to stop pain. In the case of infection, patients will receive antibiotics.

Risks of Genetic Testing: In this study, only a few genes will be tested and this test will only help doctors understand that certain therapies are better for a particular subset of patients. The results will NOT show whether the disease is caused by genetic abnormalities or linked to a particular patient. Thus patient and patient's family members will not face any problems in obtaining insurance coverage. However, in case there is possible any kind of risk to patients, or patient's family members, to keep this from happening,

the results of this test will NOT be given to anyone outside the study staff. This means that the results will not be made available to patients, patients' family members, patients' private physician, patients' employer, patients' insurance company, and/or any other party unless required by law.

Use of Genomic Testing Information: In this study, the samples will only be used for the specific regions related potential biomarkers for GBM; the results from this test will not include the full genomic information about any patient. This information from this test should NOT affect patients' insurance coverage. Insurance companies in Texas may not use the results of genetic testing to deny anyone insurance coverage, cancel their coverage, or increase their premiums. Even so, to avoid any potential risk, we will do everything possible to make sure that the results of genomic testing are not given to anyone except the study staff. The results of the testing will not be given to patients or patients' family, will not be placed in patients' medical record, and will not be given to patients' private physician, patients' employer, patients' insurance company, and/or any other party unless required by law. To further protect against the misuse of this information, patients' name will be removed from the results before the results are shared with the sponsor of the study.

Risks of Genomic Testing: The genomic tests in this study will not show that patient's disease is caused by genetic abnormalities; patients' family members will not face problems in obtaining insurance coverage for this disease. However, to keep this from happening, the results of this test will NOT be given to anyone outside the study staff. This means that these results will not be made available to the patients, patients' family members, patients' private physician, patients' employer, patients' insurance company or any other party unless required by law.

Benefits to the Subjects (if none, indicate none)

There will be no benefit to the subjects for participating in this study but future patients may benefit from this study.

Informed Consent (indicate how it will be obtained, who will obtain it, and how it will be documented; if not practicable, justify why it is not and how forgoing consent will not adversely impact the subjects' rights and welfare, included their right to the privacy of their medical records)

Taking part in this study is voluntary. All participants will be given an informed consent form and the doctors will tell the participants about any new information that may affect their health, welfare. The participants may choose not to take part or may leave the study at any time. If the individuals agree to take part in the study and then decide against it, those individuals can withdraw from the study for any reason. Deciding not to be in the study, or leaving the study early, will not result in any penalty or loss of benefits that you would otherwise receive.

Conflicts of Interest Disclosure(s) (i.e. indicate/specify any conflicts of interests and how they will be managed)

The doctor may be an investigator in this research study. If so, s/he is interested both in patients' medical care and in the conduct of this research. All participants signing up for this study or at any time during the research may discuss their medical care with another doctor who is not associated with this research project. The patients are not under any obligation to take part in any research study offered by their doctors. There are no financial interests to influence the results.

6. References

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7. Attachments/Appendices

(not included in protocol, but uploaded to the application packet in iRIS as separate documents)

- CVs of all listed on the study
- Informed consent form
- Case report forms, Data collection forms
- Recruitment materials (emails, flyers, posters)
- Questionnaires, surveys, focus group questions
- Grant (if project is funded by a grant, internal or external)
- Other research funding (departmental funding, start-up funds, etc.)
- Budget
- Others