Design Brief

Quantifying glioblastoma biomarker MGMT using fluorescence from augmented BCI*

Sujaal Gelle, Chaithra Jogiraju, Abhay Kumar Prashanth, Jagruti Lokam, Nimal Murugan, Arav Raghunathan, Naga Rajuladevi, Vaibhavee Shirkhedkar, Aarya Trivedi, Janahan Murali ▫ Lambert High School, Suwanee, GA

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Our project aims to establish a method for doctors to track glioblastoma status in patients more effectively, given the unpredictable nature of brain tumor growth. We propose a comprehensive kit comprising non-invasive and invasive components to enhance the monitoring and management of glioblastoma. The non-invasive aspect involves an augmented Brain-Computer Interface (aBCI), utilizing dry biosensors to measure EEG signals in real-time, providing insights into brain activity indicative of tumor presence. This technology offers a promising avenue for non-invasive detection and monitoring of glioblastoma, facilitating timely interventions. In contrast, the invasive component incorporates a photonic integrated chip (PIC) integrated into a chemosensor for detecting methylated O6-methylguanine-DNA methyltransferase (MGMT) gene sequences, a biomarker associated with glioblastoma. By analyzing DNA extracted from small extracellular vesicles (sEVs) in blood samples, the chemosensor employs a fluorescent DNA probe to bind specifically to methylated MGMT gene sequences, emitting fluorescence upon binding. The PIC then detects and quantifies this fluorescence, enabling precise identification and measurement of the glioblastoma biomarker. Together, these components provide a dual approach for verifying glioblastoma presence, potentially improving diagnostic accuracy. This project introduces an innovative solution with the potential to revolutionize glioblastoma diagnosis and management, offering clinicians a potentially reliable toolset for early detection and monitoring of this challenging condition.

Keywords: Glioblastoma, brain-computer interface technology, biosensor, biomarker, chemosensor

Glioblastoma (GBM) is one of the most aggressive types of cancer.. GBM, also known as grade IV astrocytoma, is a rapidly developing and severe brain tumor. It invades surrounding brain tissue but does not spread to other organs. Brain tumors are the most common malignant primary intracranial tumors of the central nervous system. Formed from abnormal star-shaped cells called astrocytes, it primarily strikes adults and has a very poor prognosis due to its rapid growth (Thakkar et al., 2019). Despite ongoing research, there is no cure, though treatment options can help manage symptoms and extend life expectancy. GBM is conventionally diagnosed through MRI imaging only after severe symptoms start to show. Sources indicate that only 1/4 of

* *The authors were mentored by Catherine Sharer and Pam Seeley from Lambert High School and Dr. Kaitlin Dailey from University of Nebraska Medical Center. Please direct correspondence to: f19723@forsyth.k12.ga.us and kadailey@unmc.edu.*

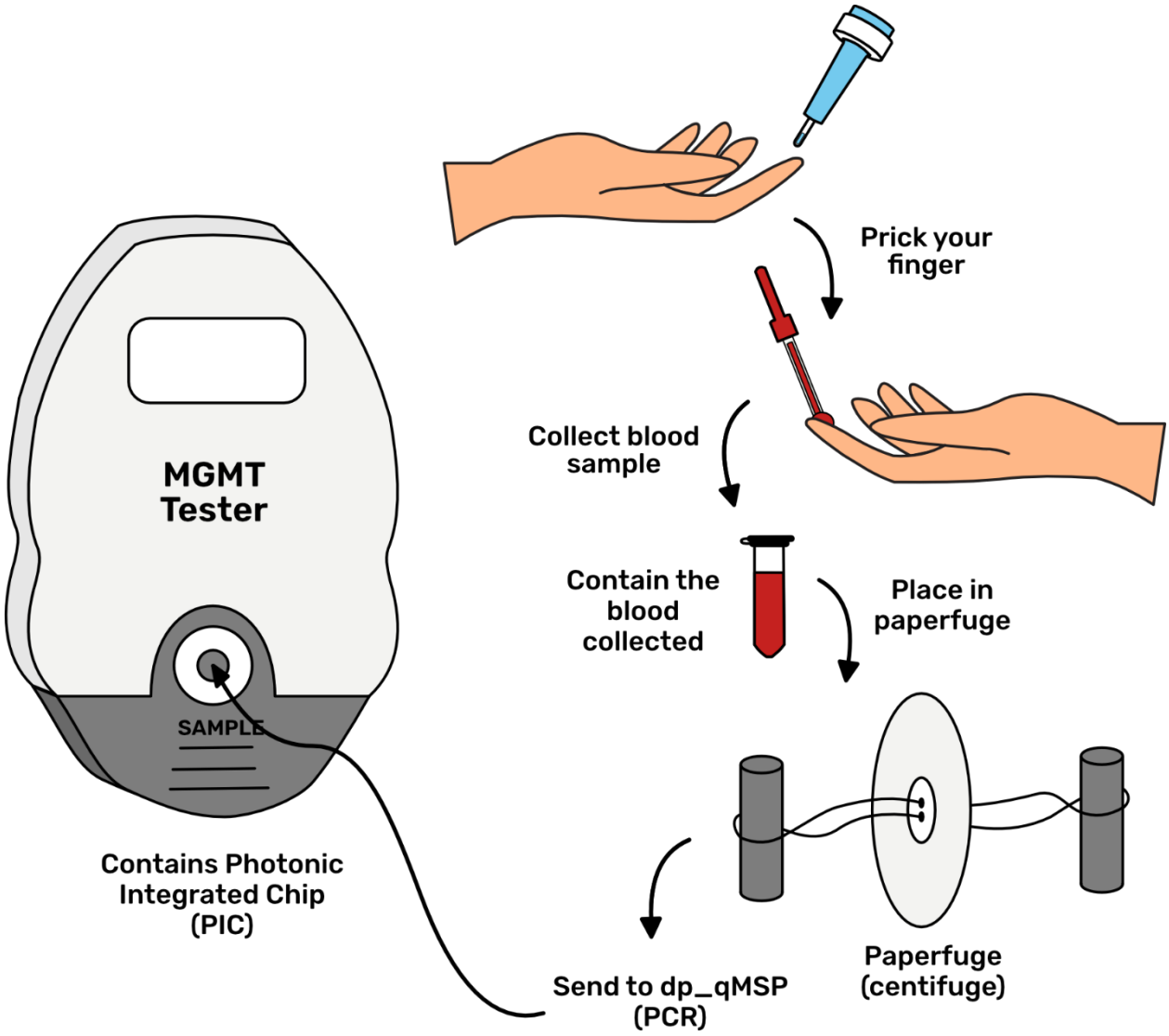

Figure 1.

individuals diagnosed with this condition survive after the first year. By detecting this condition, we can save more lives early on to prevent the fatal progression of this condition as a prevention tool (Thakkar et al., 2019).

Symptoms include headaches, seizures, nausea and vomiting, personality changes, motor skill issues, memory loss, loss of speech, and vision changes. Different treatments include surgery (extraordinarily effective and will yield tissue samples, but risks bleeding, infection, and neurological problems), radiation (can kill tissue non-invasively, but kills healthy tissue and cannot cure GBM), TMZ chemotherapy (orally taken at home and is moderately effective, but causes side effects and does not work for all patients), dexamethasone (reduces brain swelling and improves symptoms, but has side effects including immunosuppression), and specific clinical trials (offers new, effective therapies, but has no guarantee) (Thakkar et al., 2019). Our proposed solution is specifically targeted to individuals at risk of or currently diagnosed with GBM. This includes individuals with suspected symptoms, GBM history, or in high-risk groups. Hopefully, introducing our quantification mechanism will create areas for improving treatments for this deadly condition.

Systems level

The proposed solution has two components (non-invasive and invasive) for the early detection, monitoring, and management of GBM in patients.

Non-invasive components including aBCI:

The augmented BCI provides continuous real-time monitoring of brain activity using electroencephalogram (EEG) signals. The aBCI's biosensors will measure EEGs to monitor abnormal electrical signals and detect non-electroactive neurotransmitters associated with the inhibition of GBM. Our next steps will clarify how to manipulate the EEG data to collect non-electroactive neurotransmitter data. We will implement dry biosensors, which are a type of augmented BCI. The dry biosensor uses dry electrodes, which are cost-effective, placed along the scalp skin. This is more efficient as compared to a wet biosensor as it is less time-consuming and more comfortable for the patient regarding skin preparation and invasive procedures. This non-invasive component is a preliminary screening tool that can alert healthcare professionals for abnormal signals and further investigation.

Invasive components, including MGMT Chemosensor:

The chemosensor will detect fluorescence by quantifying the blood by isolating its small extracellular vesicles. Small extracellular vesicles, or sEVs, are membrane-bound, nanometer-sized vesicles that cells produce in the extracellular environment. To separate sEVs from the blood sample, a low-cost centrifuge called Paperfuge (made by Bhamala Lab in Georgia Tech) will be used. Blood must be tested for sEVs, which cause DNA to detach from blood cells and form sEV-DNA complexes to build the chemosensor. We propose to employ dp_qMSP, a specially designed quantitative real-time PCR (qRT-PCR) test capable of precisely identifying and measuring DNA molecules from the MGMT promoter region that are methylated or unmethylated. Next, we propose to employ two hydrolysis probes that have been fluorescently labeled: one that is particular to methylated MGMT DNA (FAM label) and another to unmethylated MGMT DNA (VIC brand) (Beharry et al., 2016). Subsequently, we bide our time until a response transpires and luminosity manifests. Furthermore, we utilize the photonic integrated chip (PIC) to establish contact with the solution to quantify the varying fluorescent levels, measuring unmethylated MGMT DNA. Using a formula based on the quantification cycle (Cq) values for methylation and unmethylated reactions, the test enables the determination of the proportion of methylated MGMT molecules in the blood sample.

Device level

In our home application kit for tracking MGMT, we used a reference to the A1C test used for glucose testing. To prepare the user's blood sample, we needed a finger prick.

Inspired by the A1C Test, our MGMT tester enables us to take a finger prick blood sample. Next, we take sEVs out of the blood using a Paperfuge. Our method of choice for quantifying methylation and unmethylated MGMT DNA in these sEVs is dp-qMSP PCR. The integrated photonic chip in our tester finds the fluorescent labels that are uniquely connected to each type of DNA, but

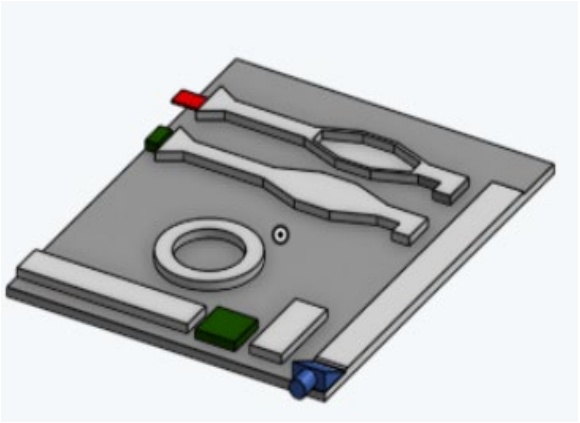

Figure 2. Sample PIC. A sample PIC that could be utilized in the MGMT tester (Raghunathan, 2024).

it only looks at the unmethylated MGMT.

Device #1: Photonic Integrated Chip / Circuit (Invasive)

A PIC is a small circuit containing electronic components that detect and handle lights. The components used on PICs, including waveguides and lasers, are designed explicitly for light-based operations. PICs can detect UPEs or Ultraweak Photon Emissions, which is essentially fluorescence on an incredibly microscopic level. Given that this incredible piece of technology can be used to detect microscale photon emissions, our team hopes to continue researching the ability of PICs to simulate BCI through fluorescence-based signals. An image of a sample model for our device is indicated below (Raghunathan, 2024)

Device #2: Dry Biosensor attached to augmented BCI (noninvasive)

The advancements of dry biosensor technology in signal processing techniques enable mobile brain imaging and novel BCI concepts that will influence many aspects of everyday life and a broad population of users. Dry-contact and non-contact type EEG sensors have been developed to improve EEG measurements, such as dry microelectromechanical system (MEMS) sensors, dry fabric-based sensors, and hybrid dry sensors. Dry MEMS sensors can perform well in measuring EEG signals when applied to the forehead or other hairless sites (Liao et al., 2009).

Parts level

Part 1: O6-methylguanine-DNA methyltransferase (MGMT)

This biomarker component is used to detect the presence of GBM tumors. The tumor cells had lower levels of MGMT. The high MGMT expression has been correlated to a decreased prevalence of GBM. However, low MGMT expression is associated with the

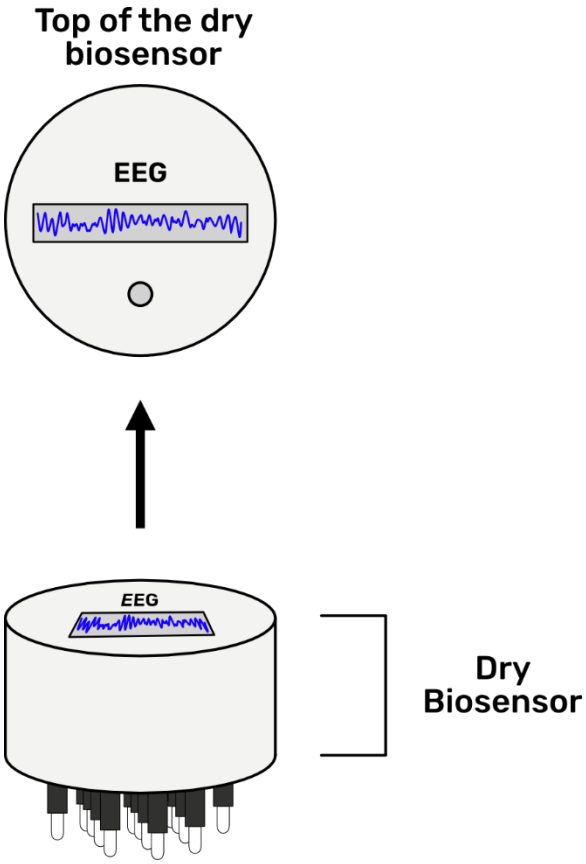

Figure 3. The second element of the kit, the noninvasive portion that is meant to be placed on a person's head, is shown in this picture. This apparatus is intended to identify EEG signals coming from the brain.

increased expression of GBM. Our sensor (PIC) will detect the presence of MGMT gene activation using fluorescence, checking for unmethylated mMGMT. If the mMGMT gene is methylated, the enzyme will not be expressed, therefore risk of GBM. MGMT expression aids with DNA repair, making it essential in the cell cycle. Any disruptions with the mMGMT (the gene coding of the MGMT enzyme) would result in unregulated cell production (Sareen et al., 2022). To be able to track the gene expression of the mMGMT gene can help to monitor and track the progress of the GBM in the patient. (Szopa et al., 2017)

Part 2: Quantitative methylation-specific PCR (dp_qMSP)

Once the sEV-DNA is separated, the DNA is

sent to a pharmacy to use MGMT-dp_qMSP which is a customized qRT-PCR that specifically detects methylated and unmethylated bisulfite-modified DNA molecules from the same chromosome location, specifically at the MGMT promoter area. The methylated MGMT would be attached to the FAM fluorochrome and the unmethylated MGMT would be attached to the VIC fluorochrome. Both probes have a non-fluorescent quencher (NFQ) with a minor groove binder (MGB) at the 3′ end. (Rosas-Alonso et al., 2021). This fluorescence will be detected by our photonic integrated chip in our kit which will help analyze chances and risks for GBM from nonfunctional MGMT genes.

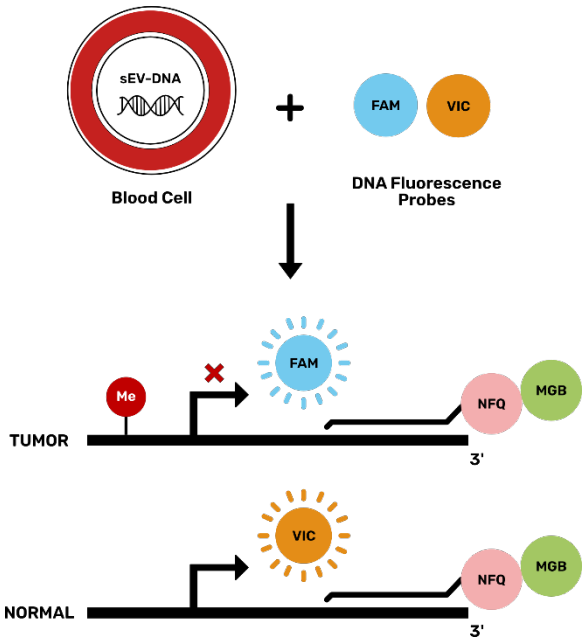

Figure 4. A diagram of both methylated and unmethylated promoter regions of MGMT genes. The two DNA fluorescence probes are combined with the sEV-DNA mixture to then bind to each mMGMT gene depending on its gene expression.

Part 3: EEGs

In our non-invasive component, the dry biosensor attached to the skin reads the EEG signals from the brain. The screen on the dry biosensor displays these signals visually. High amplitudes and crests of the waves indicated on the screen mean that a tumor, namely a GBM, is present in the brain. Normal and moderate amplitudes and crests on the waves mean the brain has no tumor. This reading from the screen would have to be verified by a health professional. EEGs were primarily used to detect focal cerebral disturbances using wave signals. However, due to the creation of MRI and CT-scan, they haven't been used for tumor detection. We decided to implement it into our kit to enhance the idea of cheaper, cost-effective ways for GBM detection (Ko et al., 2022).

Safety

Safety regarding the next steps is imperative. Patient privacy and sanitary precautions must be taken during testing to prevent infection and adverse events. All the devices used in this process should be safe for patients and researchers involved.

A BCI biosensor to obtain samples from the body, such as blood, to simulate a brain-like environment with levels of MGMT. These samples must remain uncontaminated so we will maintain proper contamination standards in the lab. Therefore, these samples should be stored at room temperature for no longer than a few hours (the true stability of MGMT will be determined after extensive experiments involving temperature and incubation period) and promptly tested. Proper lab precautions should be taken, and all researchers and doctors should maintain PPE when using the BCI on the patient. Detecting fluorescence under the presence of MGMT will also be done in the lab. Researchers should wear proper PPE when dealing with these specimens to prevent contamination.

A dry biosensor will be utilized to detect EEG signals from the brain and determine whether the patient has a glioblastoma based on them. With this, it must be known that sanitary precautions are taken. This biosensor only slightly breaks the skin, so proper precautions should be taken to prevent problems with the reading output. Infection is a critical risk with this technology, so all testing should be done in a sterile environment. Researchers should also maintain proper PPE usage when testing with

this dry biosensor.

Researchers using these technologies should be properly trained to implement the system effectively. These technologies should be used in a medical setting and operated primarily by medical professionals and researchers associated with these systems.

With BCI comes a multitude of ethical concerns. BCI should respect privacy boundaries and maintain HIPAA protocol, including mechanisms for revoking authorization. Human decision-making and autonomy should be preserved with BCI, recognizing the risk of how BCI can alter the brain by damaging nerve cells and blood cells. Proper infection precautions should also be taken to reduce the risk of infection-related illness. Informed consent should be obtained when using BCI, and all patients should be aware of all possible risks of the technology. Risk management, which involves ensuring that no treatments applied to live humans (potentially) or live animals are harmed in as minimal a magnitude as possible, should be available to keep patients secure. BCI should be used cautiously, as it is still in development, and many risks accompany it. (Richards et al., 2003)

Discussions

Some challenges seen throughout our procedure are technical difficulties of precision for people to attempt this procedure independently at home. The segregation of the sEV via the ultracentrifuge has also spurred a hassle in our project. We plan to find a way to implement sEVs in a kit that is handled in a phosphate-buffered saline (PBS) in siliconized vessels under normal conditions prior to preventing any procedure errors (Witwer et al., 2013). Another hindrance would be the qMSA implementation, which allows the patient to send their sample for amplification to a local pharmacy for accurate results, which could be time-consuming for results and may affect initial cost of the procedure. Another issue referring to the sEVs is the miniature size which could be hard to extract DNA from the blood for fluorescence therefore, our photonic chip could be proven faulty. In our next steps we refer to using a mass spectrometer to obtain better results from the sEV-DNA data collected.

The emphasis on cost-effective methods allows for GBM monitoring to become accessible to a wider population, especially in developing countries with a lack of resources. Additionally, the emergence of continuous research on early detection of GBM can pave the way for personalized treatment tailored to a patient.

MRIs are expensive and only used after severe symptoms appear; our proposed kit is designed to be more cost-effective and practical for regular monitoring in clinical settings. An MRI is not suitable for frequent monitoring due to cost and accessibility. In contrast, our kit with the aBCI system and PIC would allow for continuous monitoring with lower cost and inconvenience. Since our kit eventually incorporates a Paperfuge, which is a low-cost centrifuge that costs twenty cents. This was developed by the Bhamla Lab at Georgia Tech.

There is a high technical complexity when implementing an augmented BCI system capable of detecting EEG signals, as more penetration of the dry biosensors from the aBCI into the brain surface will reflect more accuracy of the communication of brain signals. This paper is meant to encourage the use of non-invasive aBCI technology to read EEGs for indication of focal cerebral disturbances instead of relying on expensive procedures like MRI and CT scans (Ko et al., 2022). EEG readings would help acknowledge areas of concern and associate itself with tumors which will be verified with our photonic chip sensor for MGMT gene methylation.

Infection control measures and consent with patients ensure that the safety of the researchers and patients persists. Moreover, guidelines and regulations regarding the ethical implications of BCI technology, such as patient autonomy, privacy, and informed consent, need to be established.

Future improvements that can be implemented into the system include enhanced specificity of the photonic integrated chip to detect the MGMT biomarker. This can be done by refining the chip through signal processing algorithms. Additionally, collaborating with experts from

fields such as neuroscience, oncology, engineering, and ethics can enhance the project and allow for all-inclusive solutions to the challenges of GBM diagnosis.

Next steps

The next steps to implement these ideas include optimizing the design of the dry biosensor (augmented brain-computer interface technology) to measure EEG signals and the photonic integrated chip to detect the MGMT biomarker; improvements could make the process more convenient. Convenience and automation might be greatly increased by creating a microfluidic cartridge or lab-on-a-chip device that combines sEV isolation, DNA extraction, dp_qMSP reaction, and PIC-based fluorescence detection which reduces manual handling. Investigating label-free detection methods on PICs such as plasmonic biosensors or surface-enhanced Raman spectroscopy (SERS) may be able to do away with the requirement for fluorescent probes, thus streamlining the assay. In addition to MGMT methylation studies, PICs combined with additional analytical methods like mass spectrometry may allow for thorough molecular profiling of sEV cargo.

Other refinements to the photonic integrated chip sensitivity can be done through testing and multiple modifications that may include changing the schematics of the circuit to detect fluorescence more accurately. Additionally, implementing machine learning techniques or statistical analysis methods can improve the accuracy of biomarker detection from the aBCI itself because of unreliable efficiency from sEV which are hard to detect to collect accurate data. In addition, we can establish collaborative partnerships with healthcare professionals and multiple researchers in related fields to facilitate translating augmented BCI technology into clinical settings.

To make use of the aBCI technology, it could be tested through EEG data using Natus Networks software to collect data on non-electroactive neurotransmitters. Non-electroactive neurotransmitters like glutamate play significant roles in the brain and could fluctuate normal brain activity. This could help decipher issues within communications between gliomas and neurons (Hua et al., 2022).

Author contributions

A.T., J.L., and N.R. contributed in writing the abstract. A.T., J.L., N.R., and A.R. contributed to writing the article's systems, device, and parts levels. V.S. contributed to writing the safety of the article. N.R. contributed to writing the article's discussion, next steps, author contributions, and acknowledgments. A.R., J.M., and A.T. contributed to the images. S.G., A.K.P., N.M., and J.M. contributed in writing the background.

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References

American Association of Neurological Surgeons. (2019). *Glioblastoma Multiforme – Symptoms, Diagnosis and Treatment Options* (J. P. Thakkar, P. P. Peruzzi, & V. C. Prabhu, Eds.). Aans.org. https://www.aans.org/en/Patients/Neurosurgical-Conditions-and-Treatments/Glioblastoma-Multiforme

American Diabetes Association. (2023). *Understanding A1C | ADA*. Diabetes.org. https://diabetes.org/about-diabetes/a1c

Beharry, A. A., Nagel, Z. D., Samson, L. D., & Kool, E. T. (2016). Fluorogenic Real-Time Reporters of DNA Repair by

MGMT, a Clinical Predictor of Antitumor Drug Response. *PloS One*, *11*(4), e0152684–e0152684. https://doi.org/10.1371/journal.pone.0152684

EEG in Brain Tumors: Overview, Background, Types of EEG Abnormalities Associated With Brain Tumors. (2024). *EMedicine*. https://emedicine.medscape.com/article/1137982-overview?form=fpf

Hua, T., Shi, H., Zhu, M., Chen, C., Su, Y., Wen, S., Zhang, X., Chen, J., Huang, Q., & Wang, H. (2022). Glioma-neuronal interactions in tumor progression: Mechanism, therapeutic strategies and perspectives (Review). *International Journal of Oncology*, *61*(3), 104. https://doi.org/10.3892/ijo.2022.5394

Huang, Q., Chen, L., Liang, J., Huang, Q., & Sun, H. (2022). Neurotransmitters: Potential Targets in Glioblastoma. *Cancers*, *14*(16), 3970. https://doi.org/10.3390/cancers14163970

Low-Cost Centrifuge. (2021, April 1). The Bhamla Lab. https://bhamla.gatech.edu/project-blog/paperfuge-3dfuge

Richards, H. A., Han, C.-T., Hopkins, R. G., Failla, M. L., Ward, W. W., & Stewart, C. N. (2003). Safety Assessment of Recombinant Green Fluorescent Protein Orally Administered to Weaned Rats. *The Journal of Nutrition*, *133*(6), 1909–1912. https://doi.org/10.1093/jn/133.6.1909

Rosas-Alonso, R., Colmenarejo-Fernandez, J., Pernia, O., Rodriguez-Antolín, C., Esteban, I., Ghanem, I., Sanchez-Cabrero, D., Itsaso Losantos-Garcia, Palacios-Zambrano, S., Moreno-Bueno, G., Javier de Castro, Martinez-Marin, V., & Inmaculada Ibanez-de-Caceres. (2021). Clinical validation of a novel quantitative assay for the detection of MGMT methylation in glioblastoma patients. *Clinical Epigenetics*, *13*(1). https://doi.org/10.1186/s13148-021-01044-2

Sareen, H., Ma, Y., Becker, T. M., Roberts, T. L., Paul de Souza, & Branka Powter. (2022). *Molecular Biomarkers in Glioblastoma: A Systematic Review and Meta-Analysis*. *23*(16), 8835–8835. https://doi.org/10.3390/ijms23168835

Szopa, W., Burley, T. A., Kramer-Marek, G., & Kaspera, W. (2017). Diagnostic and Therapeutic Biomarkers in Glioblastoma: Current Status and Future Perspectives. *BioMed Research International*, *2017*, 1–13. https://doi.org/10.1155/2017/8013575