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Genetic Markers and Their Medical Potential For better Brain Tumor Diagnosis, Prognosis, and Treatment

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Abstract

There is always room for improvement in medicine. In many areas, we can make strides and advancements towards better treatment, but sometimes these strides although big for those giving and receiving the treatment, can in some cases be minuscule in terms of the bigger picture of medicine. Nevertheless, these strides, no matter their size, are all essential for the overall progression. The issue that was chosen to focus on was the need for improvement in the scope of Brain Tumors. Although there is much being done, it is pertinent to focus on Brain Tumors because the tumors occur in one of the most vital organs of the body. The main issue is tumors that occur within and around the brain, and it's a vital issue to address because it is not so avidly discussed in regards to cancers that occur elsewhere in the body. Through this research we will uncover old, new, and current approaches to brain tumor assessment which directly correlate to approaching an accurate prognosis, diagnosis, and treatment plan for patients. Upon completion of finding all the aforementioned information, we will then speculate the precision and accuracy available for even better prognosis, diagnosis, treatment, etc. that could become available if those determinations were based off genetic markers commonly seen in the different types of brain tumors.

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Chapter 1

Is there a way to further assess brain tumors in a fashion that is both concise and reliable, in addition to being considerably more efficient than today's common approaches?

To find whether the question above is possible, one could propose a specific approach which could yield results that meet all aspects and requirements of a decent approach to better evaluate brain tumors that could be generalized on tumor type (through genetic makeup) or specialized to an individual's particular health (and genetic makeup). To achieve a range in specificity, this survey of literature could potentially reveal an alternative that yields a massive array of information, all of which could be put to use in achieving the overall goal which is patient survival and eradication of their tumor. With this new approach, using genetic markers could be a significant advancement in medical assessment of any patient. The primary reason to take interest into this topic is that the opportunities for progress aren't as abundant as those for cancers that occur elsewhere in the body, such as the breast or lung. This statement does not serve to invalidate or diminish the severity of any of these cancers, but more so to stress that a vital organ such as the brain isn't as replaceable or as capable of ectomies. Breast cancers have screening tests that are mandated after a certain age. These tests are considered "gold standard," which means that they are assured that all positive are positive, and all negatives are truly negative. Lung cancer has some environmental factors that are known to increase susceptibility such as tobacco smoking. These precautions and possibilities of early diagnosis aren't as readily available for brain tumors. Diagnosis of brain tumors usually does not occur unless the patient shows symptoms that merit testing. One can only conclude from this that many patients who are diagnosed, depending on the type of tumor they are diagnosed with, have to be in stages of tumor

growth that has progressed significantly without notice or warning. If this conclusion is safe to make, then it only reinforces the necessity for new approaches to brain tumor assessment. To think that there may be a potential screening tests based on genetic markers, easier diagnosis and treatment plans, or a concrete prognosis that yields a better outcome for the patient are all principal reasons as to why this idea should be considered.

A future where cancers could be completely eradicated, or even more easily treatable, is a goal to reach in modern medicine, but the path to this stage is long, full of obstacles and setbacks, and hard to find, but that doesn't mean it's not worth trying. By the end of this literature review, whether genetic markers are the possible conduits to lead medicine in the path toward this major utopian medical ideal should be proven either as a reliable pathway for the future or a goal unable to be achieved at this time.

Chapter 2-

To fully comprehend this topic, it is fundamental to define some of the major terms and concepts that will appear in this paper. Along with this background and clarification, will be the debunking of common misconceptions about cancer and brain tumors in general. This is essential as it sets the foundation of this research paper so that this information can serve as a foundation for future concepts in the coming chapters.

What is cancer?

There are two types of cancers. One is malignant, and the other is benign. The types of cancers that are benign, do not invade nearby tissue, but instead are usually encapsulated by fibrous tissue and can grow in size with the fibrous constraints. Both benign and malignant cancers can be removed, however malignant cancers are usually harder to completely eradicate since they are not as well-defined as their counterparts.

"The term cancer specifically refers to a new growth which has the ability to invade surrounding tissues, metastasize (spread to other organs) and which may eventually lead to the patient's death if untreated"(What Are Tumors 1).

This type of abnormality can occur in any part of the human body, and sometimes, depending on the location of the tumor, there aren't treatment options available. In instances such as these, patients are usually given a prognosis which may be a span of time that they are expected to live.

The cells in cancer that have the ability to grow uncontrollably and spread into other surrounding tissue or organs have multiple mechanisms that allow them to do so. There are many modes of metastasis and growth. The most common being invasion of nearby tissue (the basilar membrane or surrounding tissue). Lymphatic spread is where the cells spread to other areas through transportation in the lymphatic vessels where they can proliferate in a secondary site creating a secondary tumor. Vascular spread is when the cells spread via traveling through the veins and vessels where they can reach other organs and create a secondary or tertiary site, creating a secondary or tertiary tumor. Lastly, angiogenesis is when the cancer cells stimulate the growth of new blood vessels which create a blood supply and oxygen for the cells to continue to grow, to then cause the same issues as vascular spread. As for which path a tumor will take, that depends on the location of the tumor relative to blood and lymph vessels and nodes respectively, in addition to some other factors that will be discussed later. But it's crucial to keep note that all these modes of spreading addressed are capable only in malignant tumors, as mentioned earlier, benign tumors' capsules that surround the tumor impede the ability of the cells to embark on the malignant progressions. This information was learned in a biomedical science class taken at LIU in the fall of 2017, titled Histopathology, and instructed by Dr. Anthony Capetandes.

What are tumors?

Tumors are best defined as abnormal growth, or mass, of cells. They can sometimes be palpable depending on the location. Determining the malignancy of the tumors involves many approaches, which will be discussed in coming chapters.

The instances where the tumors are not palpable are usually because they are not in a palpable location, or because they are what is known as "diffuse". Diffuse tumors are masses of

malignant cancer cells that lack well defined perimeters and provide difficult extraction/resection complications.

Considering the organ of interest that cancer occurs in for this paper, it is evident that this type of cancer is of serious importance. A brain tumor can have many neurological effects on the body. The brain has complete electrochemical control over the body. This network within your body has a multifaceted job that one may rarely ever fully understand. To give perspective, consider your brain to be a subway system such the New York Metropolitan Transportation Authority. Now imagine a malignant tumor as a type of damage to the subway tracks in a local area such as Manhattan. On the surface of the issue, the tracks may appear rusted, and one may conclude, if the rust is removed, the subway system will be fixed and brought back to efficiency. So, the "solution" to fix the track is implemented and for a while the tracts work.

However, a couple months later the subway tracks are not responding and there are multiple delays for routes in Manhattan, but now also in Queens and the Bronx. Now there are even more delays, upset customers, larger traffic flow, and potentially more crime. This is a similar letdown that occurs when an oncologist or neurosurgeon tries to remove or destroy a tumor.

"Maintaining an organized tissue structure is an important attribute of cells making up any multicellular organism, whereas tumor cells lose this trait and take on a chaotic, highly heterogeneous pattern relative to their genetic, epigenetic, transcriptomic, morphological and biological profiles." (Baysan et al 1). This concept of organization is termed "differentiation." Each cell in the human body initiated as a stem cell, and as time progresses those cells became specialized with a given function/purpose. The cells that make up the skin offer a completely different purpose than those in the intestinal tract, or those in the kidney. This obvious

evolutionary advantage is crucial to any multicellular organism's continual growth and existence. This change occurs during gene expression, where genetic material dictates what actions a cell will take or perform.

In some instances, cells that have a high mitotic rate commonly lose heterogeneity and their differentiated function and end up becoming what is known as a neoplasm. A neoplasm is an abnormal growth of cells at an exceedingly fast rate, with malignant potential. In reference to the brain, the cells that are located here are neurons, and these particular cells generate differentiated cells called neuroblasts through a process other than mitosis. This production of neuroblasts comes from neural stem cells in the brain. These cells remain stem cells with the purpose of generating neurons and more neuroblasts. These stem cells are not found throughout the entire brain, instead they are located in specialized places, such as the hippocampus and the cerebral cortex. “Males have a slightly higher incidence compared to the females for all brain neoplasm types except for meningiomas which effects women (80%) more than men (20%)”(J. Gomes et al 82).

When a cell that has already been differentiated loses its purpose, it can become cancerous or it can go through apoptosis. In instances where apoptosis does not occur, the cell then has the potential to mutate. These mutated cells may begin to grow at uncontrollable rates. This reaction, on the molecular and cellular level, may introduce the growth of a malignant brain tumor. Nevertheless, there are still other factors of great significance to consider that may possibly increase susceptibility and risk of brain neoplasm.

Common Misconceptions

The cells of a tumor are not always uniform. This means that there can be a great difference between one cell that lies directly next to another tumor cell. This concept, is described as heterogeneity. This occurrence of heterogeneity in malignant tumors, especially brain tumors, is what makes the entire treatment process (diagnosis, treatment, prognosis, etc.) so challenging. The mutated cells that are growing uncontrollably can each have a different mutated gene.

However, common genetically mutated cells can make up the majority, of the tumor. These cells would be the primary target in order to achieve the main goal of tumor eradication. This approach leads to a new problem in treatment. To eradicate a group of cells that initially caused the proliferation of cell growth into a tumor, allows for the secondary or tertiary cells that held different mutation to grow, and often at a more extraordinary rate than the predecessor. This concept is called “recurrence,” and this occurs in many brain tumors. Clearly complete eradication of a tumor that is diffuse and heterogeneous in any location would be increasingly difficult to treat.

One would think a simple solution after eradication of a primary tumor would be to target the recurrence with a second treatment that affect the differently mutated cells in the same way as the first treatment plan. However, simplicity is not always a viable approach when tumors are of concern. The crucial goal is to alleviate the patient's illness, not create more stress or harm. To continually expose the patient to radiation or chemotherapy can result in more harm due to the cytotoxic nature of the treatments in regard to dose and frequency. These realities must be kept in mind if one truly desires to understand how to treat and diagnose a patient. This knowledge if from notes in a class that was taken for the subject of Histopathology at LIU in the fall of 2017.

Environmental and Occupational Factors

There is a plethora of suspected environmental factors that people today think induce or increase the risk and susceptibility of brain tumors (O. Idowu et al 1). In an article published by the African Health Sciences Makerere Medical School, the writers assess the common environmental factors that are believed to cause brain tumors in children. These factors include ionizing radiation, non-ionizing radiation, N-alky-Nitrosoureas, and a few others.

Ionizing radiation is a radiation consisting of particles which is implemented in techniques involving gamma rays or x-rays. In ionizing radiation, the energy that is used has the ability to destroy the nucleus of an atom. This means that techniques that have ionizing radioactive capabilities can damage DNA within the nucleus of a cell as it passes through the tissues of the body. This is why when patients get X-rays, they are required to put on a lead vest or apron to cover the other parts of their body that are not being evaluated. This lead vest reduces the body's exposure to the DNA-damaging effects of ionizing radiation. With this knowledge, it is evident why this type of radiation should be considered as a potential factor.

It is said that Ionizing radiation has a correlation to incidence of certain types of brain tumors, especially in children. “Irradiation of the cranium, even at low doses, can increase the incidence of meningiomas by a factor of 10 and that of glial tumours by a factor of 3 to 7, with a latency period of 10 years to more than 20 years after exposure”(2).

Yet, when discussing specifically X-rays and their diagnostic purposes, the article states that radiation in doses for diagnosis such a broken arm are not as harmful as their counterparts,

such as full dental x-ray. These types of x-ray are suspected to have some correlation of incidence(2). I t would behoove any parent to consider these possibilities in regard to both their children and themselves. The main contradiction of this factor is that Gamma ray radiation is used to target specific malignant cancers due to the ability to destroy the DNA of a cell. There is a procedure that exists with the sole purpose of killing cells in a selected area that uses Ionizing radiation. This procedure is called Gama Knife Radiation therapy, and since it is used in a therapeutic procedure as a way to destroy cell, it will not be included in this discussion of environmental factors that could affect brain tumor risk. However, this procedure, if enacted, brings up an ethical question as to whether or not this usage should be continued as a form of treatment, knowing that it would be targeting all cells not just the cells of the tumor.

Non- ionizing radiation does exactly what it states. Unlike its previously discussed counterpart, Non-ionizing (NI) radiation does not cause ionization through radioactivity. This is because NI radiation uses electromagnetic radiation that does not have the same amount of energy as Ionizing radiation. The types of instances where one may encounter NI radiation are near powerlines, microwaves, or infrared radiation. Radiofrequencies are also included in this list and the can be encountered daily by mobile/cellular use. However, “Recent data in humans do not support the hypothesis that the use of hand-held cellular telephones causes brain tumours ”(O. Iduwo et al 2). As for power lines, this form of NI radiation, in essence from what was read, has no evidence of contributing to increased incidence in brain tumor malignancy.

N-alkyl-nitrosoureas is a particular compound, known for its alkylating properties to DNA. It is also known to cause brain tumors in lab rats. One of the reasons this particular compound was considered a factor to be assessed for correlation of incidence is because it has the ability to pass through the placenta to the fetus. This compound can be found in meat

products and possibly contaminate water, however the studies conducted by the researchers from the African Health Sciences states that there has yet to be found any substantial results of correlation between human brain tumors and the compound.

One of the other factors that was included in this research as an environmental factor is viral infections. There have been many speculations, that some viral infections such as HIV may correlate to certain types of brain tumors in children. This has been researched extensively, yet there are no conclusive results that indicate a concrete relationship of causality between viral infections and brain tumors. Consequently, it is safe to conclude that, as of now, the principal environmental factor of concern, in its' relation to incidence and susceptibility/ risk increase, is Ionizing radiation.

As for adults, they are also at risk for the same environmental risk factors discussed previously, as well as some occupational rick factors. This list or factors in total that was discussed and assessed by The International Journal of Occupational and Environmental Medicine are

> "exposure to diagnostic and therapeutic radiations, electromagnetic radiation from cellular phones and other wireless devices, infectious agents, air pollution and residence near landfills and high- voltage power lines and jobs as firefighters, farmers, physician, chemists and jobs in industries such as petrochemical, power generation, synthetic rubber manufacturing, agricultural chemicals manufacturing."(J. Gomes et al 82).

In the article authored by J. Gomes et al, the environmental and occupational factors have substantial research which indicates that there may be a correlation between certain occupations and an increased risk of primary brain tumors (specifically intracranial malignant gliomas,

astrocytomas, and meningiomas). Consult Appendix B for the occupation and the suspected level of risk due to exposure.

Other not so common occupations that were mentioned by J. Gomes et al are those in hazard cleanup and those that work with/near metals. Jobs where workers were introduced to arsenic and mercury were correlated to brain neoplasm incidence increase. Occupations with hazard cleanup saw a slight increase in incidence of brain neoplasm in those who worked on the nuclear radioactive cleanup of Chernobyl (92). Evidently occupational and environmental risks are not as clear cut. The subject area of brain tumors and their incidence through outside factors contains a large gray area. The principal goal of future research into this topic will most likely center around finding a gold standard outline to each brain tumor's cause, treatment, prognosis and diagnosis.

Exclusion of certain factors are due to limitations such as small population size; differences in parameters of assessment in exposure in terms of latency, exposure time, etc.; age, ethnicity, co-exposure, and more. However, this does not negate or invalidate the aforementioned findings for correlation with primary brain tumors. The best approach would be to minimize the limitations uncovered by J. Gomes et al by exploring studies that find possible effects on an almost universal or standardized basis. These would need to have parameters the could produce results that are reproducible and accurate, with group of the same tumor type, similar age, and social/economic factors.

What are genetic markers?

Molecular information like this offers solid evidence that can be securely used without much room for doubt. “Genetic markers are variants in the DNA that are associated with a

specific disease phenotype revealing variations DNA Marker technology has revolutionized the world of genetic research… A marker may have functional consequences, such as altering the expression or function of a gene that directly contributes to development of disease." (S. Raza et al 221). Chiefly put, genetic markers are like guide maps that can help a doctor better figure which tactics to employ when treating or diagnosing a patient, and this is made possible due to their expression, suppression, or location relative to other genes.

Whether it is multiple mutations, single mutations, whole chromosomal gains or losses, or even wild type genes, any of these genetic markers could be used. Scenarios where physicians cannot agree on issues involving their patients' health or uncertainty in any biological test can possibly be swayed if specific, individual molecular and biological information is used as a map to concise information for the patient and their health.

Chapter 3

Classifications of Brain Tumors

Early Classification

The World Health Organization (WHO) is an entity that represents information found by a committee of specialized doctors and surgeons that consult on criteria used for the determination and assessment of particular diseases. In addition to this, WHO also applies these evaluations to new findings and information. The conclusion made by the members are used on a universal basis (but not mandated to be used), so that when a patient is diagnosed there are not any discrepancies or confusion on why or how they were diagnosed with a specific brain tumor. For consistency, only classification systems from the WHO classification of tumors of the Brain Central Nervous System will be used. This is compiled of the information proposed from 117 contributors from 20 different countries in the 2016 edition, in addition to all previous editions of the classifications to illustrate to the reader the progress that has been made over the years. (D. Louis et al 2)

The history of the classification system for brain tumors starts in 1979. The first edition of the WHO classification was published that year and it assessed how to determine what brain tumor a patient should be diagnosed. This work matured over the years and offered numerous notable advancements that are now put to use in many aspects of tumor assessment around the world. This particular edition did not offer as much as it progenies, but it's significance still stands as the first step of many that helped oncology and medicine reach all the accomplishments that have been achieved so far (B. Scheitauer 1-4)

In the early 1990's , the second edition of the WHO classification was published, and like all new editions, new information such as types of tumors and variants were introduced. At this time, the use of this classification system was still optional. The only well-known approach to tumor classification was based on histopathological appearances, and these classified tumors formed a short list that included some of the most significantly assessed tumors as of recent publications, such as different astrocytomas and meningiomas. Different, meaning in their morphological appearance regarding to shape, color (density of color), and the rate at which the cells proliferate.

In this histopathological approach a new technique of assessment was introduced. Tumors such as the "monstrocellular sarcoma" , were renamed on the basis that the "immunohistochemica1 studies clearly showed that tumour cells consistently express GFAP" (P. Burger et al 262). GFAP stands for "Glial fibrillary acid protein." It is a common protein that is found in cells in the central nervous system such as astrocytes and ependymal cells. GFAP can be detected by immunohistochemistry. In immunohistochemistry, antigens are imaged by using known biological assets (antibodies) of the tissue selected which will bind to the antigens. This is done so that once the binding has occurred, the antibodies which can be seen under a microscope will indicate what ailment the patient has. This technique uses markers specific to the particular disease to help determine what cannot be decided based on a macroscopic basis (Immunohistochemistry Principle). This change in classification based on a molecular parameter is significant because it illustrates the major blind spot that was in medicine before its implementation.

Early Tumor Grading

Guidelines were also introduced that detailed which characteristics of tumors should be seen in order to grade the level of malignancy. Some of these characteristics included requirements and observations such as proliferation rate (whether it be fast or slow), ability to invade nearby tissues, necrosis and atypia. The third edition was published in 2000, and differs from it's to ancestors in that it has additions such as predictive features of new tumor types, along with descriptions of the entities in clinical, radiological, and other biological aspects (D. Louis et al 215). This edition contains the major turning point for brain tumor classification. The inclusion of genetics as a defining feature in naming tumors has finally come, and with its introduction, the products that were generated led oncology down a sturdy path to better overall tumor assessment.

The WHO grading system in this edition was set forth as a guide to depict the level of tumor malignancy. For instance, this excerpt from The Journal Neuropathology and Experimental Neurology below discusses the genetic content that lead to the changes in grading of certain tumors.

> "Astrocytic brain tumors span a wide range of neoplasms with distinct clinical, histopathological, and genetic features. Molecular genetic data that has been gathered since the prior WHO classification in 1993 suggest that individual histologically defined types of astrocytomas are even more diverse at a biological level"

In addition to this discovery, they found that glioblastomas are characterized by amplification of a particular gene, in addition to some other special proteins expression and suppression. These attributes helped label the glioblastoma as a primary grade II brain tumor. For secondary glioblastomas the criteria applied to their grading were the occurrence of TP53 mutations that

arose in over half of their cases , and in over eighty percent of their cases for anaplastic astrocytomas. (219).

The most noteworthy additions to the new edition are the newly recognized mutations and deletions that gave enhanced insight into the tumor on a more structural level. Chromosomal changes are mentioned, indicating that there's great potential behind the use of these parameters in aspects other than classification. This article speculates the strength and practicality that this genetic information could offer in terms of clinical approaches, and even treatment plans. It is pertinent to acknowledge that the editions preceding this third edition, although not mandatory to be used, shows the continual effort put in bettering the patient and doctors understanding of brain tumors, and possible causes or commonalities among tumor types, and that should be admired.

Recent Tumor Grading

The 2007 publication discussed more generalized parameters that offered a further breakdown of the classification system. These new attributes that led to this accomplishment is the addition of information such distribution of occurrence in age or gender. Location of the tumor and the way the tumor progresses during clinical trials were also factored into this system. This new edition had contributions from a plethora of individuals and countries that collaborated to aid in furthering the usefulness of this classification system so that it can be used on a universal basis at one's discretion.

Examples of these parameters' enactment include the change of Pilomyxoid astrocytomas from its original grading to a WHO grade II, based on its common patient distribution among

infants and children as stated in the article, in addition to its poor prognosis. There is also the use of CNS as a prefix when naming a particular subtype of tumor, specifically CNS neuroblastomas or CNS ganglioneuroblastomas, due to neuronal differentiation or the presence of neoplastic ganglion cell respectively (D. Louis et al 10).

One of the elements that stayed the same through this 2007 edition was the format used for grading brain tumors. A complete breakdown of what qualifies each type of tumor from grade I to grade IV is depicted below (10).

> For Grade I, the cells have a low proliferation rate, and there is a possible potential of curing the patient through removal.
>
> For Grade II, these tumors are understood to have some notable infiltrating and proliferation potential, and a possibility of recurrence. Some Grade II tumors can even progress into high grades.
>
> Grade III tumors are known to be malignant capabilities, high proliferation and cell division, and commonly require chemo and radiotherapy.
>
> Finally Grade IV tumors, which is the worst grade of all in terms of prognosis, known for pre and post disease evolution and fatal outcomes, are seen to have necrosis in surrounding tissue, extreme malignancy, and widespread infiltration and possibly dissemination.

After 2007, there was almost another decade before the fifth edition was published in 2016. In this edition of the WHO classification system, a table that lists all the changes that were made from the previous editions depicting new variants, tumor types, histologic or genetic

findings, etc., along with a table of their current grading system for a select number brain tumors was included. (see appendix C)

In respects to classification, the breakdown of how malignancy grades of brain tumors are simple to understood, but this should not be mistaken as the universal breakdown for all tumors. In actuality, to determine the malignancy grades that can be given to a tumor, first the tumor itself must be classified, and other tumors similar to it identified. This is done by first looking at the location of the tumor. Particular tumors have common location within the brain that they occur such as the cerebellum for medulloblastomas, or in the posterior fossa for ependymomas. Then, the next aspect to consider in grading is the size of the tumor, and whether its looks concise with clear borders or diffuse with unclear indication as to where it starts and end. After determining these macroscopic characteristics, one can then embark on the microscopic and molecular parameters to assess the tumor. This will consist of immunohistochemical assessment for common marker that indicate proliferation rate, mitotic activity, and more information to determine the level of malignancy. With these finding, this could help differentiate between a tumor that may appear to have a clear prognosis, but highly malignant and aggressive cells on the molecular level that indicate high potential of invasiveness or mortality; meanwhile another tumor that appears diffuse at the macroscopic level can present immunohistochemical data that indicates low recurrence rate after resection. It is because of this that malignancy grades in tumors are specific for each tumor type.

Nomenclature

In addition to the latest provisions, the newest edition of WHO classification included an acknowledgement of progressive information that was withheld from the 2007 edition. This information was not included due to the unknown plausibility of this information to stand alone as its own factor instead of a subcategory or aspect of a larger condition or component in the classification process; A breakdown of how the names are written; common challenges and obstacle faced; and new variants are present in all new editions.

D. Louis et al stated that the combined use of phenotypic and genotypic parameters heightened and cleared the missing aspects of diagnosis. With this approach, which now include genetic information which could also be called genetic markers, could theoretically provide improved accuracy in diagnosis, patient management, and prognosis of brain tumors and cancers.

As for the nomenclature , the names follow a setup similar to most classification systems, where there's a hierarchy to what order the information should be mentioned for each type of tumor. Most tumor classifications have the histopathological name with the genetic features following after a comma. One example given in the text is "*diffuse astrocytoma, IDH-mutant."* If there were multiple genetic features, then they were both included in the name. If there was a lack of genetic information due to unattained molecular diagnostic testing, then some tumors received the designation "NOS" which stands for, "not otherwise specified." (D. Louis et al 11)

Nevertheless, with progress, there's always a new obstacle to address and resolve. This issue in the recent edition was labeled as "discordant results," or in other terms, conflicting results. When histological results contested with molecular genetic results, there was the issue of deciding which piece of information should be used, and which should be disregarded, in order

to properly name particular tumors. The decision to use genetic information when there was conflict with results solidified the efficiency of genetic markers' capability and strength in brain tumor research advancement. Certain brain tumors from this classification of a tumors in the central nervous system will be used as focal points to show support for the use of genetic markers in the discussion on what should be done if there's a way to readily identify specific tumors. In doing this, an explanation as to whether the assertions that were made can be validated, in order to exemplify the indisputable weight that genetic markers could carry in medicine.

What to do with new findings?

With this clarification comes another aspect that should be addressed. This issue is the malignancy of the entire tumor. Until now, it may have been presumed that the whole of the tumor mass is malignant and dangerous. This would be a precarious assumption to make, but to avoid any discrepancies or confusion, its pertinent to state that the malignancy of a tumor is usually determined by a focal point of the mass, as found by V. Collins. Because there is a specific section of the tumor that expresses characteristic of malignancy, these sections should be assessed in detailed in order to give the proper treatment.

Toward the conclusion of the article for the 2000 edition of WHO classification, P. Kleiheus et al states that the opportunity of "… assessing whether a tumor with a particular molecular feature responds to a specific therapy may provide important information about choosing among currently available treatments and for the design of future therapies, regardless of whether any prognostic information is provided." (P. Kleiheus et al 223). Added to this was

the discernment that the new entities that came about as a result from genetic typing helped to establish gene expression patterns that could be used to easily predict an individuals' response to different types of therapies and treatments (223).

Essentially, a full tumor assessment should be made before treatment or therapy courses are taken. Consider having a load of laundry that one is in a rush to clean, and in that load is a red sweater that can ruin all the other clothes. Associate the importance of separating your laundry and compiling all the proper colors into the correct piles as the same steps that need to be taken in assessing a tumor before treatment can be implemented.

Information such as patient history is similarly important. It should be understood that brain tumors have a multifaceted complexity, and that all information as to lifestyle, environmental factors and occupational factors is necessary in order to classify the tumor, but also to treat the tumor.

The implications of this new standard approach should consequently aid in deciding prognosis, treatment and therapy, and, in some instances, clinical trial selection. A "neuropathologist may be expected to make a diagnosis on the basis of often very small and fragmented biopsies" (V. Collins et al 1), and it is crucial to take the patients and patients' family medical history into consideration in order to make the best diagnosis , in addition to collaborating with other medical professionals, as a collaboration would offer better insight than a single doctors' findings.

The brain tumors that most commonly occur for adults are diffuse astrocytic tumors, oligodendrogliomas and meningiomas, while pilocytic astrocytomas, ependyomas, and medulloblastomas are commonly found to occur in children(V. Collins et al 1). A synopsis of

information similar to this, and on the aforementioned tumor types that can be used as a reference point, illustrating the information from the different editions classifications systems and other sources can be found in appendix E. When reading the coming chapters, this synopsis along with the other figures can serve as elucidations to offer a better understanding as to why brain tumor approaches, prognosis, treatment, and diagnosis are different for each tumor.

Current Assessment of Brain Tumors

There are multiple ways to observe a brain tumor and its effects on the patient. The options available are MRI, Tissue sampling/biopsy, CT scans, Positron emission tomography (PET) Scan, Cerebral , arteriogram/angiogram, Lumbar puncture/spinal tap, Myelogram, Neurocognitive assessments, and Electroencephalography (EEG). However, the only approaches that will be assessed in this thesis are MRI, CT-scans, Tissue sampling, and Lumbar puncture/spinal tap.

For MRIs, the primary information that could be concluded is tumor size, location and enhanced imaging. “Some studies suggest that tumor location affects survival and may play a role in prognosis” (N. Mickevicius et al 394). An article published by The journal of Neurooncology, discussed the capability of viewing brain tumor intersecting white matter as a way to predict patient prognosis. Through MRI, this article discussed whether total or subtotal/partial resection of tumors would be the best route for treatment based on MRI images that showed tumor cell migration along the path of white matter tracts.

The results of this approach to tumor treatment and prognosis through use of MRI showed crucial information about tumors in regard to the tissue they invaded, their location, and other finding correlated to drug treatment. To comprehend the coming material see **Appendix F.**

With these findings, it obvious that MRIs do offer great insight into brain tumor treatment and prognosis. And when combined with other approaches, this could yield a highly plausible tactic to better treat brain tumor patients and give more accurate prognoses.

When considering which treatment course to take, the entire tumor and even seemingly unaffected areas around it are all of great importance. Medical imaging procedures and techniques are used to view these areas to help give oncologist a better idea of how to treat an individuals' particular brain tumor. One of these techniques is the computed tomography, or CT scan.

CT scans create images which help to decide dose level of a particular treatment path a patient should be prescribed. N. Mickevicius et al found that CT scan slice thickness, in relation to tumor size, does have a correlation as to which treatment plan and dose a patient should have. The size and planes the CT scans are taken in can range from 1, 2, 4, 6, and 10 millimeters, and can be in the sagittal, coronal, axial, and arbitrary view respectively.

Corresponding article, by R. Caivano et al found the same conclusion when conducting a study with a spherical mass which "showed that for targets less than 1.5 cm in diameter, it is reasonable to acquire CT images with the smallest thick- ness available. They also recommended, for conformal radiotherapy treatment planning, 4 and 8–10 mm CT slice thickness for targets between 1.5 and 3 and for targets greater than 4 cm in diameter, respectively."(R. Caviano et al 507). Both N. Mickevivius et al and R. Caivano et al essentially found there is an optimal slice thickness in tumor treatment planning for tumors or particular sizes. It is findings such as these that may seem simple, but in actuality take a significant amount of time to compile the necessary data and information to make such solid assertions.

When using techniques such as CT scanning, information is provided to an oncologist and whomever they collaborate with interprofessionally when consulting with a patient on treatment plans. However, the specificity and detailed needed for differences in tumor size in relation to slice thickness and angles show that this option, although helpful, is difficult. This technique can be seen as exceptionally trying when the patient has to go through multiple imaging sessions to find the appropriate slice thickness and angles to their particular brain tumor.

For lumbar puncture and spinal taps, cerebrospinal fluid (CSF) is used to determine if the DNA found in this fluid offered any insight to the brain tumors in the patients. The motivation behind this was that cerebral spinal fluid can be assessed for genetic material such as Cell free DNA. In the article that discusses this medical approach, written by Wenying Pan et al, the method of this technique included the sample being taken and amplified using polymerase chain reaction (PCR) with targeted amplicon sequencing to increase and concentrate the important DNA found within the "liquid biopsy".(W. Pan et al 2).

In this approach, the scientist found that they were able to detect tumor mutations within the CSF from Cell-Free DNA, and that these samples included both primary and metastatic brain tumor mutations from the pool of the seven patients they assessed. The DNA found in this experiment is called Cell-Free DNA, meaning that it was found outside of cells in the CSF from a tumor. This type of DNA is particularly hard to extract, hence the need for PCR to increase the quantity of DNA found so that it can be efficiently analyzed. The suspected reason for the difficulty is that the blood brain barrier may hinder the movement of the Cell-Free DNA to travel through the body.

This is of significant importance because it demonstrates the great potential of tumor content assessment without the invasive approach which requires collecting brain tissue that has been affected by a tumor. This ability to detect mutations led to the innovative advancement to "characterize tumor mutations in CSF for potential clinical diagnosis"(2). The information derived from the mutations were the detection of known driver mutations, which were capable of allowing the researches in this article to keep watch over brain metastasis; and "global characterization of genomic aberrations to direct personalized cancer care." (3).

Driver mutations are mutation that drive the malignant spread of a tumor. These genomic aberrations are created as a byproduct of the breakdown of the machinery used in DNA replication. These aberrations are classified as duplications, deletions, translocation, and other genetic based changes (M. Guttman et al 1). One strategy assesses focal points of mutations in CSF, and the other strategy used by Wenyin Pan et al, assess genomic aberration through panel sequencing with known DNA aberrations found in cancer genes. These strategies lead to the conclusion that brain tumor mutations are conveniently easier to detect in cerebral spinal fluid than in plasma (W. Pan et al 2).

The conclusion of this article for assessing CSF weight in tumor treatment and prognosis is explained in the excerpt below.

> "The first strategy, an approach based on digital PCR and targeted amplicon sequencing, is cost effective and highly sensitive. … For patients who have had tumor resections, their tumor mutations can be identified from the surgical sample with standard clinical tests, such as sequencing or genotyping arrays. …The tumor types that most commonly metastasize to the brain, such as breast, lung, and melanoma, have well characterized

hotspot mutations amenable to this strategy. The second strategy… has potential to guide personalized cancer therapy, monitor residual disease, and track the evolving tumor genome ”(9).

Clinical Approaches to Brain Tumors

Clinical trials are used to test potential agents and therapeutic substances to determine whether they are successful enough and efficient enough to be administered to patients on a larger scale. M. Gilbert published an article titled, Facing the Future of Brain Tumor Clinical Research. In this article, the clinical trials that were assessed were cytotoxic chemotherapy agents, signal transduction modulators, biological agents, antiangiogenic agents, and immunotherapies .In addition to the types of substances used, the method of introduction of the substances into the patients was also considered. The Studies researched showed low efficiency in treatments that used drug capable of crossing the blood brain barrier, so alternative options that used direct injection, implantation of slow release vehicles, and other approaches were used instead (M.Gilbert et al 2).

The issues that consistently hindered clinical approaches were believed, to stem from inherent heterogeneity and continual genomic transformation of the cancer, the integrity of the blood brain barrier, and unique microenvironments consisting of glial cell, microglia and other cell types(5). Other issues mentioned were the size of the population diagnosed with the particular tumor type being assessed; or patients who do have the diagnosis of the desired tumored being too far in progression, unwilling to participate, or no knowledge of clinical trials available (4).

These clinical trials and approaches are well-intentioned, and some do offer great advancements. The way this progress in the trials is assessed is through patient health, categorized as Overall Survival (OS), Progression-Free survival (PFS), and Response Rate (RR). The FDA has declared the evidence of symptomatic benefits is a meaningful criteria as a measure of clinical benefit as well. The system used to check the symptoms each patient has is a list of 23 items where the patients are asked to describe how the symptoms affect them on the daily and the severity of their symptoms. Its hoped that there will be progress made on a better system as this seems to be too narrow of a spectrum for symptoms patients feel or don't feel.

Clinical approaches to date still have major advancements sets before them. This does not invalidate or discredit the value of the information that has been found, but serves to filter to the audience that information found here still needs work. Circling back to the point made earlier that everything will continually need improvement as we go through time, to recover a potential path towards what could be the next significant approach in medicine for brain tumors would be an astounding achievement.

Chapter 4

Drawbacks

The drawback from using MRI technology is that this technique, along with the others, will not be used until the patient expresses symptoms similar to those expressed in people who have brain tumors. This technique has significant use, but this is mostly the case when it combined with other procedures, and not so much when its' on its own. MRI can help diagnose brain tumors, and can be useful in determining patient progression, but the need for improvement can never be fulfilled.

To achieve better results for patients with brain tumors, there needs to be a technique that can offer universal or multifaceted applications to keep pace with the multifactorial complications found in each type of brain tumor. These aforementioned techniques of MRI and CT scan imaging can help with this development, but they are not capable of being the face of process. Spinal tapping/lumbar punctures are seemingly good perspectives as ways to achieve and overall better patient assessment of an individuals' tumors, but this task also needs to be further studied to assure its reliability.

These procedures are still significant, and should continue to be used, but the face of this golden approach should be genetic markers. The information and resources cited in this paper had one common factor, which was the inclusion of common genetic phenomena. These phenomena were genetic mutations, common chromosomal changes, genetic expression such as GFAP, and more. These phenomena were seen in the classification system, the diagnosis of tumors, and the predictive prognosis of patient health and treatment.

Plausibility for Genetic Markers?

One of the common pathways that are altered in tumors is the process of programmed cell death. For Apoptosis this pathway called the p53 pathway, is commonly mutated in carcinogenic cells of brain tumors. The gene TP53 regulates the cells of the body, and when it's time for a cell to die, this pathway will be enacted. But with this gene is mutated, the original capability of the gene to stop proliferative growth and other tumor suppressive attributes are destroyed.

The information published by G. Fulci et al on p53 and its relation to brain tumors was found in 1998. These findings showed that genetic markers use has been essential for decades in better understanding tumors (G. Fulci et al 1)

When planning treatment courses, the genetic make-up of certain cells in a heterogeneous tumor mass can be assessed, to pick treatment plans that can target all the cells based on their genetic expressions. And, in instances where tumor heterogeneity leads to recurrence, this too can probably be concluded by assessment of the primary tumor and the genetic expression found in it relative to the secondary tumors.

As for patient prognosis, in the classification system, there were tumors that are known to have poor prognosis based on previous cases where mortality was within months of diagnosis, but if a genetic parameter could be used to screen for this type of malignant growth/cell type, then prognosis although not positive, would still be well founded in cases where the range of months could differ from 6 to 8 moths in difference per each brain tumor type of high malignancy and mortality.

In an article published by the Journal of Neuropathology & Experimental Neurology, J. Roth et al uses the genetic change of an entire chromosomal gain and its commonality in relation to the possibility of a brain tumor return after a surgical removal. The tumors used in this assessment were Pilocytic astrocytoma (PA) and a variant of it. The researchers conducted a retrospective analysis of the information attained from patients who were diagnosed with PA from the years 1998-2014. The patient size in total for this research was 116, with 122 tumor specimens.

In the experiment the researchers discovered a 2.0 M.b. gain on chromosome 7 in a band named 7q34, and that this genetic change resulted in the KIAAI1549-BRAF fusion. The fusion occurred in 74% of the 116 patients they had specimens from. Other sightings included different chromosomal changes such as gains for the chromosomes 5,6,11,15,and 120. The relationships between co-occurrence of each type of genetic changed mentioned were also assessed (i.e. Chromosome 7 gain with *BRAF* fusion, or *BRAF* fusion with other chromosomal gains), but these statistics were insignificant as the percentages of co-occurrence was low, and showed no correlation to information on tumor recurrence. Through assessing every aspect of this information and other statistics mentioned in the article, the researchers concluded that chromosome 7 gain showed a significant increase risk of recurrence than those patient specimens that did not have chromosome 7 gain. Through the analysis of the genetic changes present in patient specimens, researchers were able to conclude a prognosis of PFS, and possibility of recurrence. (J. Roth et al 2)

The international Journal of Cancer did a similar assessment on glioma stem cells to determine if any genetic changes offered any significance to an increase in the possibility of recurrence. In this study by M. Baysan et al, the specimens used are glioma stem cell lines (both

polyclonal and monoclonal) from an initial tumor diagnosis, along with tissue samples from the same patients' recurred tumor after they deceased. Unfortunately, one of the findings in this article states that in terms of comparison between the initial tumor specimen and that of the recurrent specimen taken after death showed no common mutations. However, this seemingly significant blow to the use of genetic markers was completely evaded. It turns out that the reason the common genetic mutations found in the initial tumor sample were not found in the recurrent tumor was because the treatment plan to eradicate the initial tumor was successful in the eradication of the tumor mass by killing a targeted cell type within the tumor; and because this eradication was successful, the cells that took over the proliferative properties of the initial tumor were able to grow and spread, causing the recurrence.

> "It would appear that in this particular patient, selective pressures presumably from radiation, various cytotoxic and antiangiogenic agents succeeded in eliminating the original high-proliferating clone that dominated at disease presentation. Subsequently, however, there was an emergence of a distinct invasive clone that predominantly localized to the corpus callosum and contralateral hemisphere. Although most glioblastoma transiently respond to initial therapy, tumor recurrence is inevitable and ultimately leads to the patients' demise."(M. Baysan et al 12)

The outlook for patients may seem to be grim, but the need for advancement still lives. In the section where the researchers discuss genomic results, the authors found that a particular homozygous mutation of focal P16 deletion and homozygous p53 mutation were found consistently in all their samples, and correlated that these same genetic markers were seen in "Homozygous focal p16 deletion and homozygous p53 mutation were among the well-maintained events across all our samples. Moreover, these events are highly recurrent in GBMs

in general"(8).Although the search for common mutation between recurrent and initial tumors was unsuccessful, the common mutation of any GBM were still found, thereby endorsing the value of genetic markers.

New information found through the assessment of chromosomal gains and losses of chromosome 7 and 10 respectively, were speculated to indicate that as time passes, the buildup of mutations can indicate the "clonal age for the monoclonal GSCs" (10) in instances where mutation accumulate over time. In addition to this, the researchers concluded through a whole overview of the data and information found, that it is strongly advised to re-biopsy a patient with GBM before they treat with any targeted therapies, since the finding that illustrated new tumor cells in a recurrent tumor will need a new approach to destroy the newly dominant cells (12).

Conclusion

From the data, statistics, experiments and other information presented in this work, it's evident that Genetic markers offer a promising approach to tumor classification, prognosis, and treatment. The hypothesis is this paper was to determine whether Genetic Markers are capable of being used as screening test for brain tumors, along with their ability to aid in a better prognosis, diagnosis, clinical trial set up, and treatment plans for patients.

This paper clearly demonstrated that genetic markers are already being used in collaboration with many other techniques to continue the medical advancements in neurological and oncological research. The coming tables illustrate the common mutations found in all the tumors mentioned throughout this paper; the percentage at which each mutation occurs; the uses each mutation can be applied (i.e. diagnosis, prognosis) (S. Park et al 6-7).

The tumors that were discussed have been underlined for easy detecting in tables 4 that can be found under **Appendix G**. For common mutations consult table 5, under **Appendix H**

As for screening test, there were not any true screening test for brain tumors in patient who had not already had an initial tumor removed, or expressed symptoms that correlated to brain tumors. It's a fair speculation that this has not been done yet because the idea may seem invasive; or that the plethora of mutations to look through may offer more complexity than solutions in a screening examination. Nevertheless, the option should be further explored, similar to that of the clinical approaches mentioned in Chapter 3, that took permission and certifications to propose the clinical trials.

The hope is that, there can be a computer system where one can input all the significant mutations of the most commonly researched tumors, and screen spinal fluid to detect any possible DNA alterations that may indicate the presence of tumors. This test could be optional, for people to take at their leisure, or mandatory for everyone, and covered by insurance companies, like mammograms to screen for breast cancer. The test that are mandated could be regulated based on the environmental and occupational factors that are likely to increase risk/susceptibility of brain tumors, and those who live or work in instances that correlate to the instances mentioned in this paper would be required to take screening tests at set interval of months or years depending on age, to assure population health.

Other ways to create proper screening techniques would be creating profiles that include patient history and family history, this would be one step in the right direction towards better targeting patient needs which give clarity to doctors as to how to better approach overall brain tumor assessment. This proposal, will obviously take some time before it can be implemented, the

necessities of this type of procedure for screening would need permission, specimens, data, and consent from patients and nation originations such as WHO, NIH, CDC, and others. In addition to these safety parameters, there would need to be a computer system with the ability to take a surplus information and assess correlations and commonalities among specimens and patient information, with the ability to disregard repetitive findings. This system should also have a self-checking parameter encoded so that errors, such as incorrect matching of information, or incorrect correlations to brain tumor susceptibility and mutations can be avoided in assessment. Generally, this concept still needs work, but it is certainly applicable given the right foundations of support. Therefore, the hypothesis of genetic markers capabilities is validated. They offer a large magnitude of information in better treatment, diagnosis, and prognosis. The further the field of oncology proceeds in the use of genetic markers as a way to tackle the big monster that brain tumors are, the more likely we will be able to better treat and help alleviate the pain and burden inflicted on patients by brain tumors.

Appendices

Appendix A

Overall survival (OS)- A term to describe how long a patient will survive post diagnosis or post treatment.

Progression-Free Survival (PFS)- A term to that assesses how long a patient will survive without recurrence/regrowth of a tumor after resection.

Magnetic Resonance Imaging (MRI)- A medical imaging technique that uses radio waves and a computer to generate pictures of areas inside the human body.

White matter (WM)- Sections of the brain tissue that is pale (white color appearance), consist of nerve fibers, located between the gray matter which makes majority of the peripheral tissue of the brain.

Computed Tomography scan (CT)- A medical technique that uses multiple x-ray images/pictures attained from different angles at different "slice" thickness, combined with a computer to generate images that offer anatomical views of organs and tissues in depth.

Polymerase chain reaction (PCR)- A technique used in molecular applications which can amplify single copies or segments of DNA.

Response rate (RR)- The rate at which a patient responds to a particular treatment

Apoptosis – this is programmed cell death, where the cell itself enters the pathway that enables it to destroy itself due to damage.

Necrosis- cell death that is random, and not programmed. Could be caused by cell damage

Atypia- This term describes cells that appear atypical or abnormal from the original morphological appearance.

Prognosis- The prediction or forecast of the likely course of a disease and patients' mortality.

Diagnosis- The identification of a disease.

Polymerase Chain Reaction (PCR)- A laboratory technique/protocol that analyzes a short sequence of DNA, through amplification of targeted DNA through the use of probes and amplifiers.

Metastasis- The spread of a malignant growth in a secondary phase, either in surrounding tissue or other organs.

Appendix B

- Pesticide applicators
    - A suspected three-fold increased risk due to agricultural chemicals
- Petrochemical refineries
    - A proposed increased risk of gliomas for workers in petrochemical industry is believed to be the result of exposures to a number of occupational factors. (chemicals such as organic solvents, aromatic acids, plastic monomers or
- Synthetic rubber manufacturing
    - "exposure to synthetic rubber products is reported to increase the risk of glioma.[31] Exposures to plastics are reported to increase glioma risk"
- Firefighters
    - Marginally increased risk
- Veterinarians
    - Increased risk
- Electricity generation workers

- Glioma, but not meningioma risk was associated with the duration of job involving exposure to electric and magnetic fields. The risk increased with the duration of the exposure (p=0.05 for trend). The risk was greatest for astrocytoma (OR=4.3; 95% CI: 1.2–15.6).

The reader should note that the work done by an electrical engineer exposes them to electric and magnetic fields, and that in the article published by The African Health Sciences Makerere Medical School , focused on exposure to children and correlation to incidence, while The International Journal of Occupational and Environmental Medicine assessed the correlation of increased risk and incidence in adults through occupational and environmental perspectives.

Appendix C – *Table 1*

Formulating concept of how CNS tumor diagnoses are structured in the molecular era

Major restructuring of diffuse gliomas, with incorporation of genetically defined entities

Major restructuring of medulloblastomas, with incorporation of genetically defined entities

Major restructuring of other embryonal tumors, with incorporation of genetically defined entities and removal of the term “primitive neuroectodermal tumor”

Incorporation of a genetically defined ependymoma variant

Novel approach distinguishing pediatric look-alikes, including designation of novel, genetically defined entity

Addition of newly recognized entities, variants and patterns IDH-wildtype and IDH-mutant glioblastoma (entities) Diffuse midline glioma, H3 K27M–mutant (entity) Embryonal tumour with multilayered rosettes, C19MC-altered (entity) Ependymoma, RELA fusion–positive (entity)

Diffuse leptomeningeal glioneuronal tumor (entity) Anaplastic PXA (entity) Epithelioid glioblastoma (variant) Glioblastoma with primitive neuronal component (pattern) Multinodular and vacuolated pattern of ganglion cell tumor (pattern) Deletion of former entities, variants and terms

Gliomatosis cerebri

Protoplasmic and fibrillary astrocytoma variants

Cellular ependymoma variant

"Primitive neuroectodermal tumour" terminology

Addition of brain invasion as a criterion for atypical meningioma

Restructuring of solitary fibrous tumor and hemangiopericytoma (SFT/HPC) as one entity and adapting a grading system to accommodate this change

Expansion and clarification of entities included in nerve sheath tumors, with addition of hybrid nerve sheath tumors and separation of melanotic schwannoma from other schwannomas

Expansion of entities included in hematopoietic/lymphoid tumors of the CNS (lymphomas and histiocytic tumors)

Appendix D- *Table 2*

WHO grades of select CNS tumours

Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	II
Anaplastic astrocytoma, IDH-mutant	III
Glioblastoma, IDH-wildtype	IV
Glioblastoma, IDH-mutant	IV
Diffuse midline glioma, H3 K27M–mutant	IV
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	II
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted	III
Other astrocytic tumours	
Pilocytic astrocytoma	I
Subependymal giant cell astrocytoma	I
Pleomorphic xanthoastrocytoma	II
Anaplastic pleomorphic xanthoastrocytoma	III
Ependymal tumours	
Subependymoma	I
Myxopapillary ependymoma	I
Ependymoma	II
Ependymoma, *RELA* fusion–positive	II or III
Anaplastic ependymoma	III
Other gliomas	
Angiocentric glioma	I
Chordoid glioma of third ventricle	II
Choroid plexus tumours	
Choroid plexus papilloma	I
Atypical choroid plexus papilloma	II
Choroid plexus carcinoma	III
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	I
Gangliocytoma	I
Ganglioglioma	I
Anaplastic ganglioglioma	III
Dysplastic gangliocytoma of cerebellum (Lhermitte–Duclos)	I
Desmoplastic infantile astrocytoma and ganglioglioma	I
Papillary glioneuronal tumour	I
Rosette-forming glioneuronal tumour	I
Central neurocytoma	II
Extraventricular neurocytoma	II
Cerebellar liponeurocytoma	II
Tumours of the pineal region	
Pineocytoma	I
Pineal parenchymal tumour of intermediate differentiation	II or III
Pineoblastoma	IV
Papillary tumour of the pineal region	II or III
Embryonal tumours	
Medulloblastoma (all subtypes)	IV
Embryonal tumour with multilayered rosettes, C19MC-altered	IV
Medulloepithelioma	IV
CNS embryonal tumour, NOS	IV
Atypical teratoid/rhabdoid tumour	IV
CNS embryonal tumour with rhabdoid features	IV
Tumours of the cranial and paraspinal nerves	
Schwannoma	I
Neurofibroma	I
Perineurioma	I
Malignant peripheral nerve sheath tumour (MPNST)	II, III or IV
Meningiomas	
Meningioma	I
Atypical meningioma	II
Anaplastic (malignant) meningioma	III
Mesenchymal, non-meningothelial tumours	
Solitary fibrous tumour / haemangiopericytoma	I, II or III
Haemangioblastoma	I
Tumours of the sellar region	
Craniopharyngioma	I
Granular cell tumour	I
Pituicytoma	I
Spindle cell oncocytoma	I

Appendix E- ***Table 3-***

Brain tumors common in children:

Medulloblastoma

- Medulloblastoma has a peak incidence in childhood but also can occur into late middle age.
- Histologically childhood and adult medulloblastoma are identical, being highly cellular, malignant invasive tumours corresponding to WHO malig- nancy grade IV.
- Medulloblastomas occur in the posterior fossa. They consist of densely packed tumour cells with round to oval or carrot shaped hyperchromatic nuclei with scanty cytoplasm, high mitotic and apoptotic rates, and usually neuroblastic rosettes in some areas.
- The most common chromosomal abnormality in medullo- blastomas is iso-chromosome 17q, in which most of the short arm is lost from two chromosomes 17 and they are then fused head-to-head producing a chromosome with two centromers, little 17p and two 17q arms. This is observed in 30–50% of cases by using cytogenetic techniques .

Ependymoma

- The most common location is in the fourth ventricle, followed by the spinal canal, lateral ventricles, and the third ventricle.

- Children have the highest incidence of ependymomas, but they can occur into late middle age.

- There are a number of subtypes. The least biologically aggressive are malignancy graded as grade 1, and consist of the subependymoma (intraventricular and often symptomless) and myxopapillary ependymoma that most commonly occurs at the cauda equina.

- The tumour named ependymoma is malignancy graded as grade II and has a number of histopathological variants. Ependymomas show in some area(s) evidence of an ependymal cell phenotype—by the formation of ependymal rosettes and sometimes canals

- These findings have been confirmed by molecular genetic data that have identified losses on 6q, 9p, 10, 11q, 13q, 17p and 19q.

Pilocytic astrocytomas

- Patients with pilocytic astrocytomas that can be excised have a good prognosis.

- Pilocytic astrocytomas are generally biologically non-aggressive and are remarkable among astrocytic tumours in maintaining their grade I status over years and even decades (in contrast to the diffuse astrocytic tumours in adults).

- Pilocytic astrocytomas show a wide spectrum of morphologies, from the pilocytic, bipolar cellular areas with Rosenthal fibers (fig 1) to less cellular protoplasmic astrocytoma-like areas with eosinophilic granular bodies and clear cells.

- Pilocytic astrocytomas most commonly occur in the cerebellum of children. However, they may occur anywhere from the optic nerve to the medulla oblongata.

Brain tumors common in adults

Meningiomas

- Meningiomas are usually solitary lobulated tumours arising in the meninges and attached to the dura. They are believed to develop from meningothelial (arachnoidal) cells, despite the fact that the meningothelial form is far from the most common.

- Symptomatic meningiomas represent 13–26% of primary intracranial tumours, are most common in middle aged and elderly patients, and show a pronounced female predominance.

- meningiomas may progress from grade I tumours to tumours of higher malignancy grade. This is associated with losses on chromosomal arms 1p, 6q, 9p, 10p and q, 14q, 18q, as well as gains and some amplifications on many other chromosomes

Oligodendrogliomas

- Oligodendrogliomas occur mainly in the cerebral hemi- spheres of adults.

- They are believed to derive from oligodendrocytes.

- Increases in nuclear pleomorphism and hyperchromatism, as well as pronounced hypercellularity, brisk mitotic activity, prominent microvascular proliferation, and/or spontaneous necrosis, results in a picture that is histologically classified as anaplastic oligodendroglioma (malignancy grade III).

- Oligodendrogliomas show relatively specific genetic abnormalities that differ from the other gliomas. Loss of genetic information from 1p and 19p was demonstrated in a genomic wide analysis in 1994[92] and this was later linked to a good response to PCV treatment, an association that is currently under intense scrutiny as it provides the first molecular indicator of treatment response in brain tumours.

- The losses on 1p and 19q are most common among the grade II oligodendrogliomas (reports of up to 90%) and are present in over 50% of anaplastic oligodendrogliomas (malignancy grade III).

Diffuse astrocytic tumors

- The adult diffuse astrocytic tumours include the astrocytomas (malignancy grade II), the anaplastic astrocytomas (malignancy grade III), and the glioblastomas (malignancy grade IV).

- The astrocytoma malignancy grade II tumours have a peak incidence between 25 and 50 years of age, while the glioblastomas have a peak incidence between 45 and 70 years.

- All are more common in males and most are located in the cerebral hemispheres.

- Anaplastic astrocytomas (malignancy grade III) show increased cellularity but the tumour cells still show histological and immunocytochemical characteristics of astrocytes.

- Cytogenetics, CGH, and molecular genetic techniques all show that the losses of alleles on 6q, 13q, 17p and 22q, as seen in the astrocytoma malignancy grade II, occur at similar or higher frequencies in the anaplastic astrocytomas.

- In addition to the genetic abnormalities resulting in the disruption of the p53 and Rb1 pathways, over 90% of glioblastomas lose alleles from 10q. The regions consistently lost include the variously named PTEN/MMAC1/TEP1 tumour suppressor gene at 10q23–24.

Appendix F

- VSA revealed that patients with tumors intersecting the right anterior thalamic radiation (ATR), right inferior fronto-occipital fasciculus (IFOF), bilateral corticospinal tracts (CSTs), and corpus callosum (CC) had decreased OS com- pared to patients with tumors intersecting WM tracts else- where

- Interestingly, age turned out to be a factor with tumor location. A large cluster in the posterior left parietal lobe was associated with younger patients, while a smaller subset of clusters in the right frontal lobe was associated with older patients.

- Tumors intersecting the right ATR, right IFOF, right and left CST, and CC were associated with decreased OS.

- Tumors intersecting the CST, body of the CC, right ATR, posterior IFOF, and IFL are associated with decreased PFS, while tumors intersecting the right genu of the CC and anterior IFOF are associated with increased PFS.

- Subjects who had bevacizumab have a significantly improved survival prognosis if their tumors intersect the ATR, IFOF, CST, or CC.

Appendix G- *Table 4*

	WHO grade	Altered genes	Genomic alteration (%)
Astrocytoma	II, III	*IDH* mutation	65
		P53 mutation	96
		ATRX mutation	96
Glioblastoma	IV	*EGFR* amplification	57
		PDGFRA amplification	13
		EGFRvIII mutation	20
		PTEN homozygous deletion	25–35

	WHO grade	Altered genes	Genomic alteration (%)
		CDKN2A homozygous deletion	61
		BRAF V600E mutation	1–2 (epithelioid GBM)
		TP53 mutation	25–35
Oligodendroglioma	II, III	*IDH* mutation	100
		1p/19q codeletion	100
		CIC/FUBP1 mutation	56/29
		TERTp mutation	80–96

	WHO grade	Altered genes	Genomic alteration (%)
Subependymal giant cell astrocytoma	I	*TSC1* and *TSC2* mutation	100
Pilocytic astrocytoma	I	*BRAF-KIAA1549* fusion	75 (cerebellar tumor)
		BRAF V600E mutation	13–15
Pleomorphic xanthoastrocytoma	II, III	*BRAF* V600E mutation	66
		CDKN2A homozygous deletion	50
Angiocentric glioma	I	*MYB-QKI* fusion	100
Ganglioglioma	I, III	*BRAF* V600E mutation	25

	WHO grade	Altered genes	Genomic alteration (%)
		TSC1 and *TSC2* mutation	Unknown
Craniopharyngioma, papillary type	I	*BRAF* V600E mutation	100
Craniopharyngioma, adamantinomatous type	I	*CTNNB1* mutation	100
AT/RT	IV	*SMARCB1* deletion/mutation	> 95
	IV	*SMARCA4* mutation	< 5
Cribriform tumor		*SMACB1* deletion/mutation	100
Meningioma, fibrous type		*NF2* inactivation mutation (no hot spot)	45–55[50]

	WHO grade	Altered genes	Genomic alteration (%)
		TRAF7/KLF4 mutation	100 (secretory meningioma)
		AKT1 (p.Glu17Lys) mutation	2.5
		SMO (p.Trp535Leu) mutation	5
		TERTp mutation	10
Langerhans cell histiocytosis	I, III	*BRAF* V600E mutation	50–57[54]

Appendix H - *Table 5*

	Target probe	Control	Cut off	Indication	Biomarker
1p deletion	Chr1p36	1q25	1p < 0.8 and Deleted nuclei > 50%	ODG	Diagnostic, prognostic, and predictive
19q deletion	Chr19q13	19p13	19q < 0.8 and Deleted nuclei > 50%	ODG, HGG	Diagnostic, prognostic, and predictive
BRAF gain	Chr7q34	CEP7	Gold signal > 3	PA	Diagnostic, prognostic, and predictive

	Target probe	Control	Cut off	Indication	Biomarker
CDKN2A (9p21.3) homozygous/hemizygous deletion	Chr9p21.3	CEP9	HoD ≥ 10% HeD ≥ 50%	HGG	Diagnostic and prognostic
EGFR amplification	Chr711.2	CEP7	Ratio ≥ 2.0	HGG	Diagnostic and prognostic
PTEN HoD /HeD	Chr10q23.31	CEP10	HoD ≥ 10% HeD ≥ 50%	HGG	Diagnostic and prognostic
RELA-C11orf95 fusion	Chr11q13.1	CEP11	Break apart	S-ependymoma	Diagnostic and prognostic

	Target probe	Control	Cut off	Indication	Biomarker
C19MC amplification	Chr19	CEP19	Ratio ≥ 2.0	ODG, GBM	Diagnostic and prognostic
SMARCB1	Chr22q11.23	CEP22	NF2 < 0.8	AT/RT	Diagnostic and prognostic

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