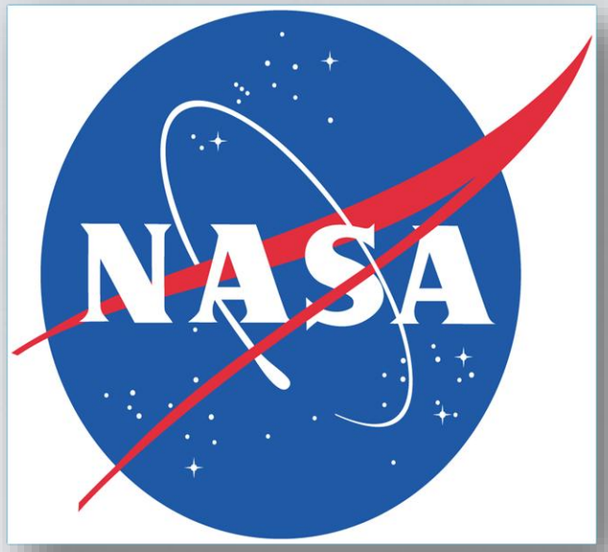


Special thanks to the NJ Space Grant NASA Fellowship for their support.

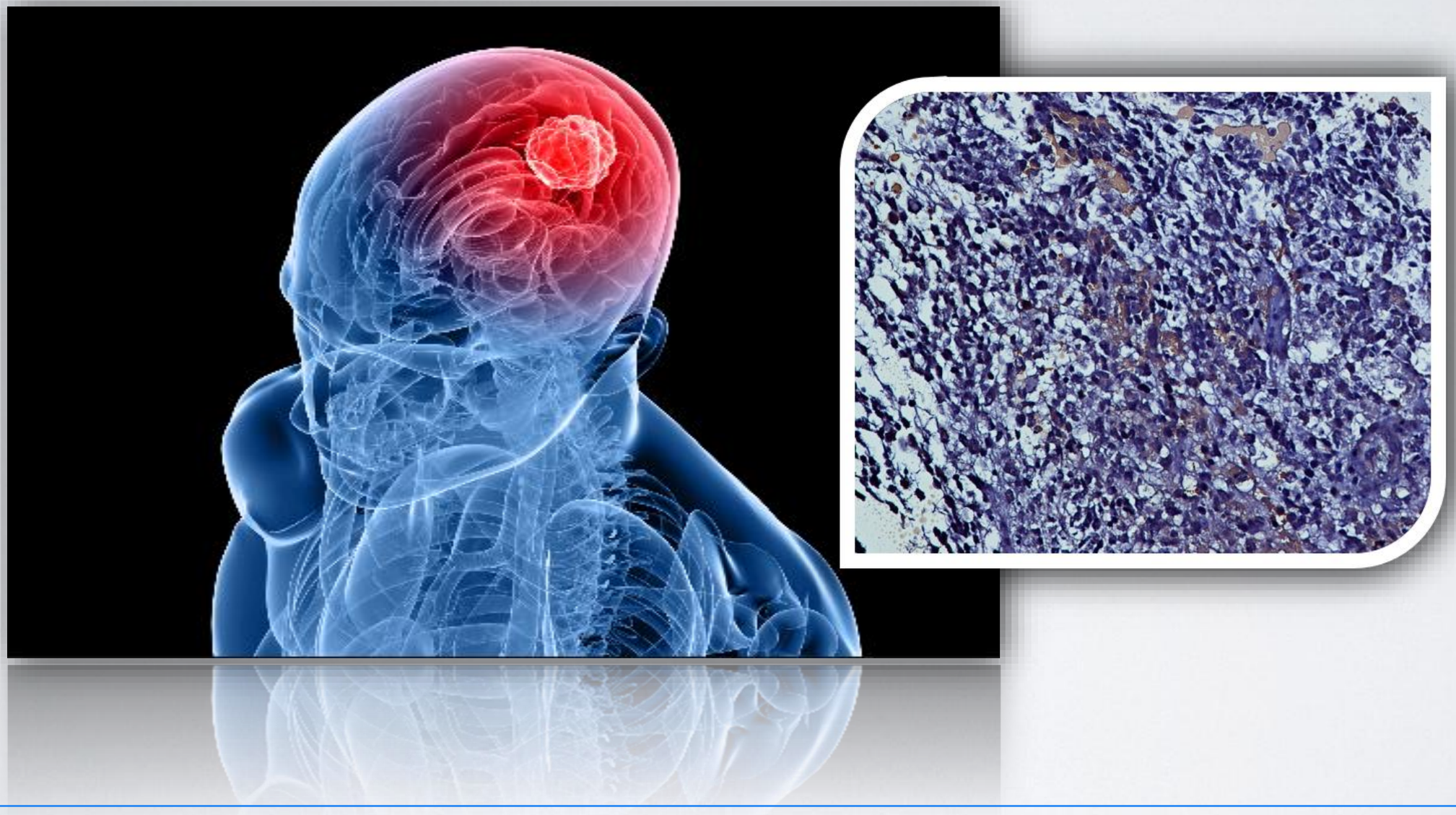
Danielle Caruso

Middlesex County College

J.F.K. Medical Center

Additional thanks must be given to the J.F.K. Medical Center Neuroscience Institute where all of my research was conducted.

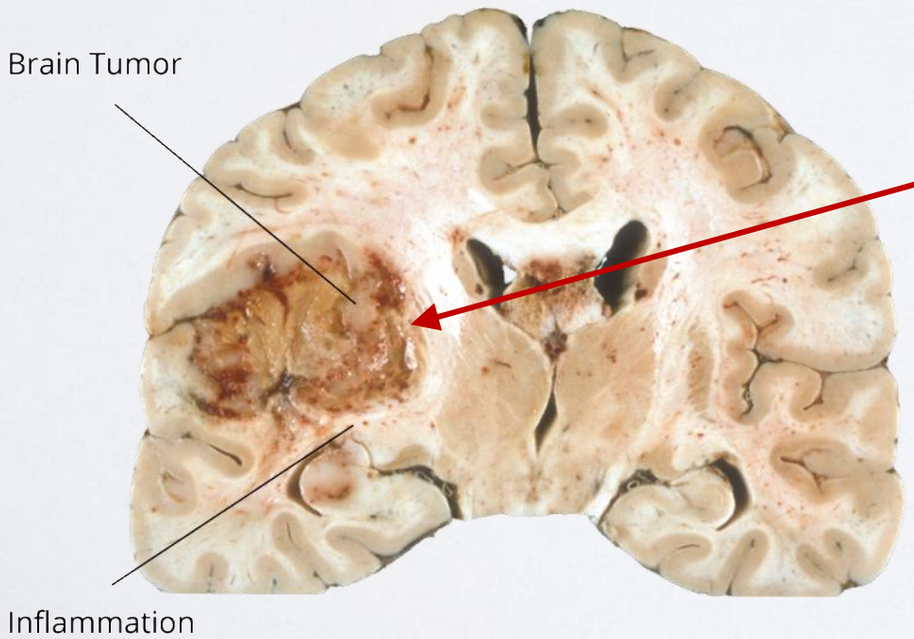
A Look at TLR4 and MyD88 in Glioblastoma Using Immunohistochemistry



Presented by: Danielle Caruso
Middlesex County College

What is **Glioblastoma**?

Glioblastoma is a form of brain cancer.



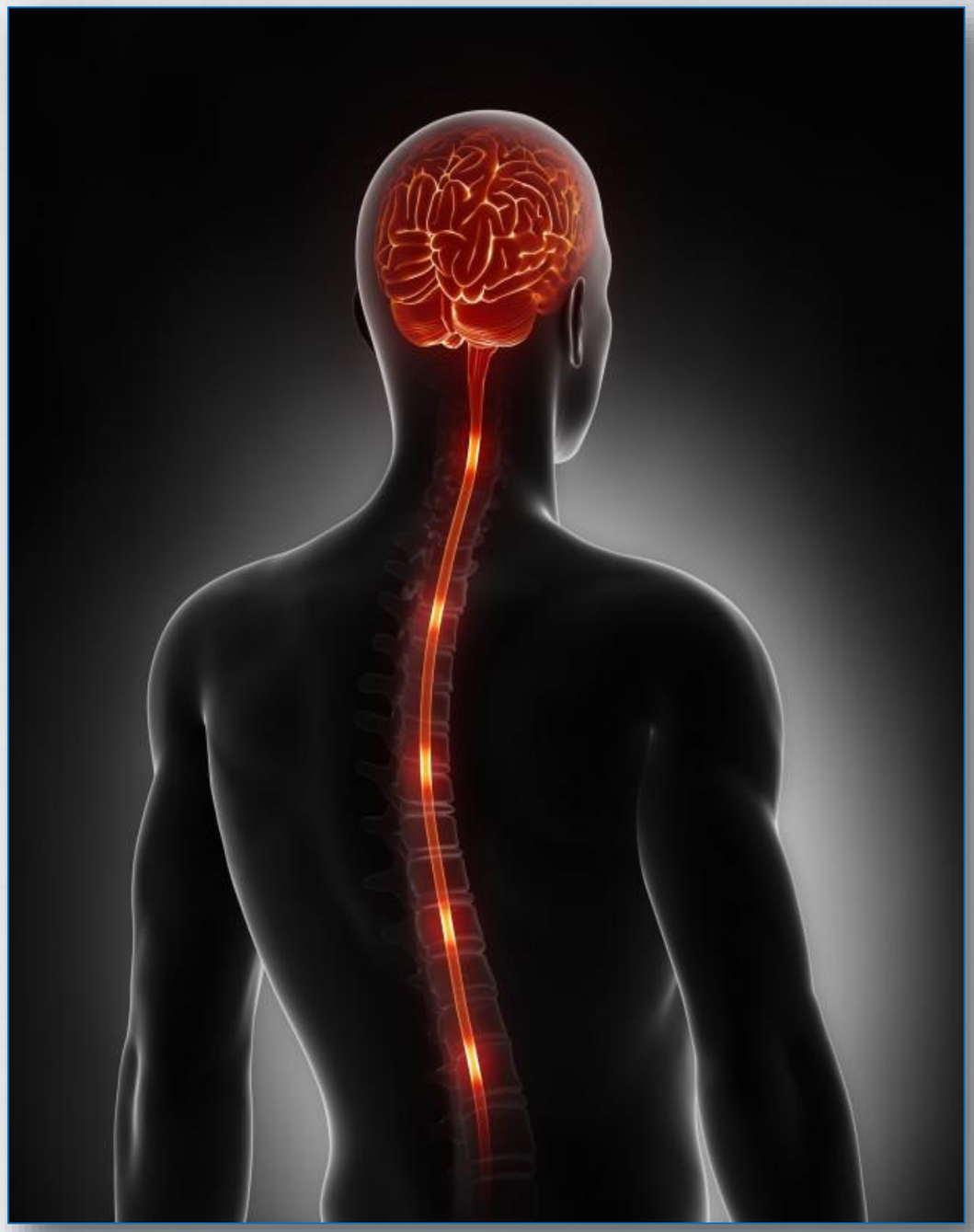
Central Nervous System (**CNS**)

- The complex of nerve tissues that controls the activities of the body

- Brain and spinal cord

- Brain is made up of **neuroglia** (supportive tissue of the CNS)

- Consists of two cell types:
  - **Neurons** (nerve cells)
  - **Glia** (connective tissue of the nervous system)



Function of Neurons

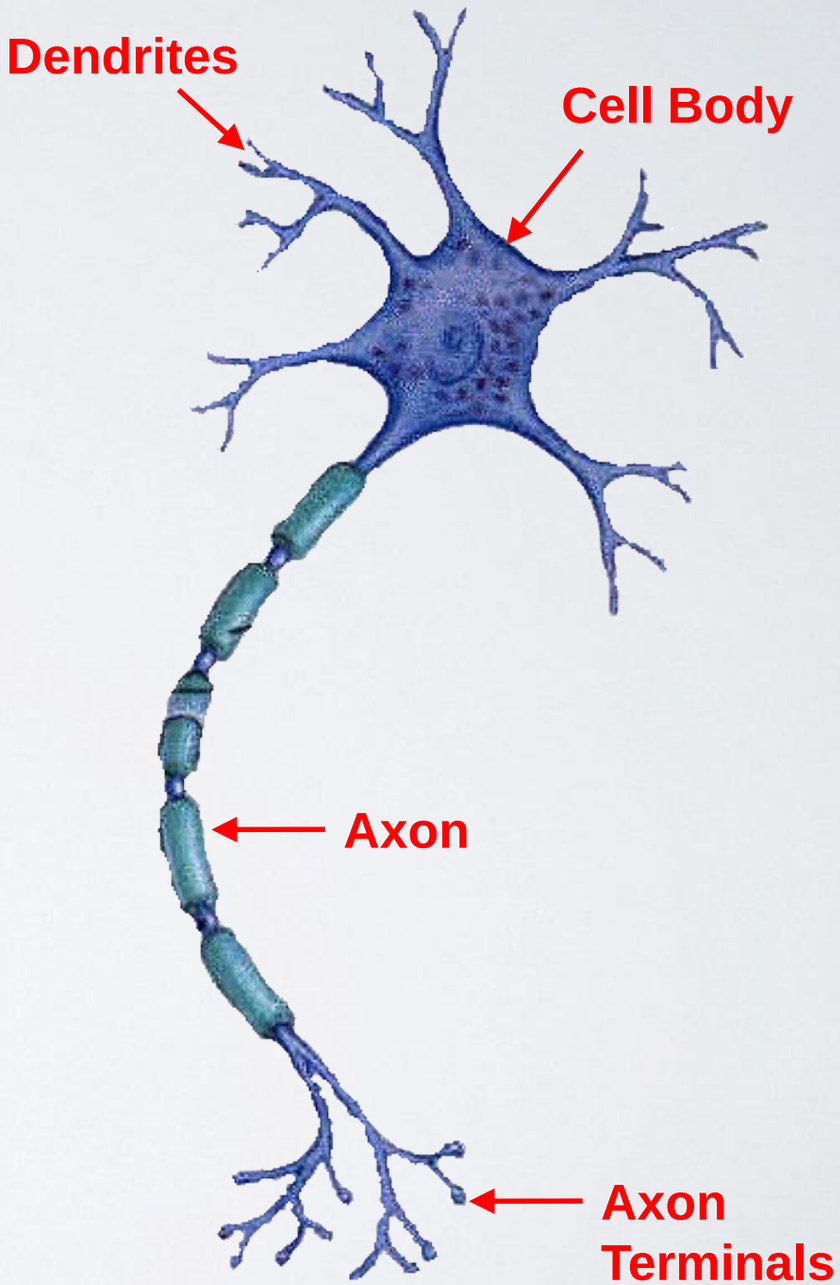
- Neurons process information

- A neuron is made up of four major parts: the cell body, the axon, the dendrites, and the axon terminals.

- The cell body (soma) contains the nucleus of the cell

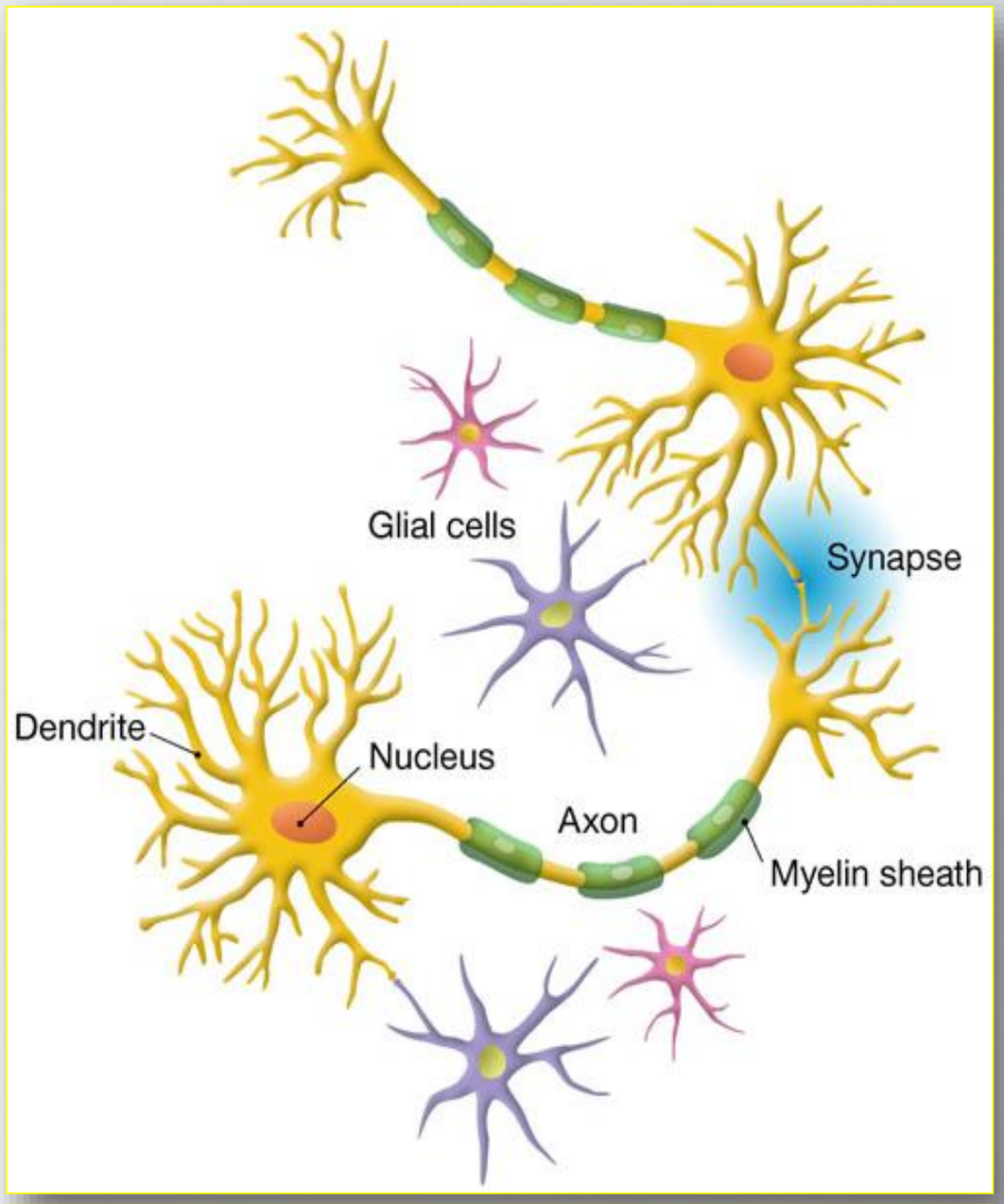
- The axon carries information from cell to cell

- The dendrites receive the signal from other cells via the axon terminals



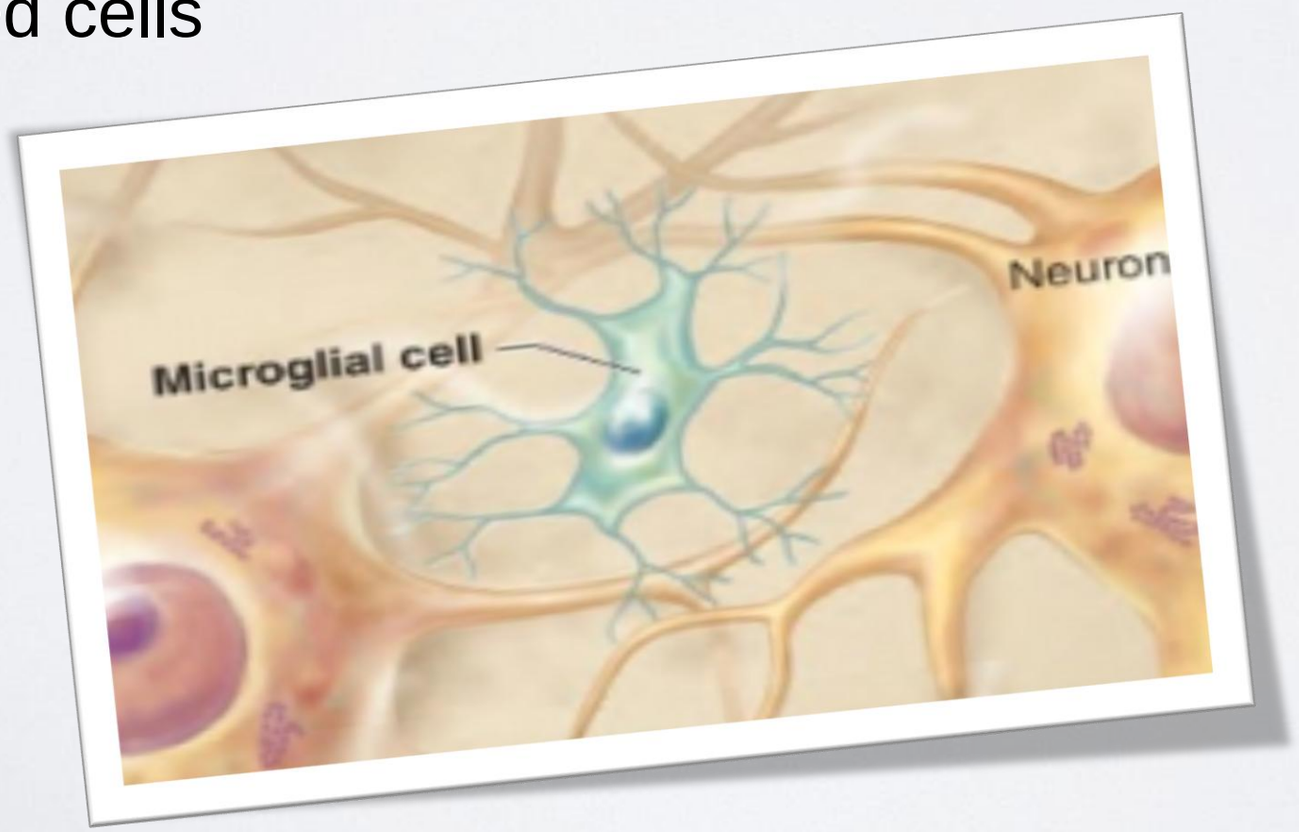
Glial Cells

- Glia hold the brain together
- Found in the space between neurons
- There are two types of glial cells:
  - **Microglia**
  - **Macroglia**



Microglia

- Primary immune cells of the CNS
- Activated when brain is infected or injured
- Phagocytose (engulfs) and destroys pathogens and/or damaged cells



Macroglia

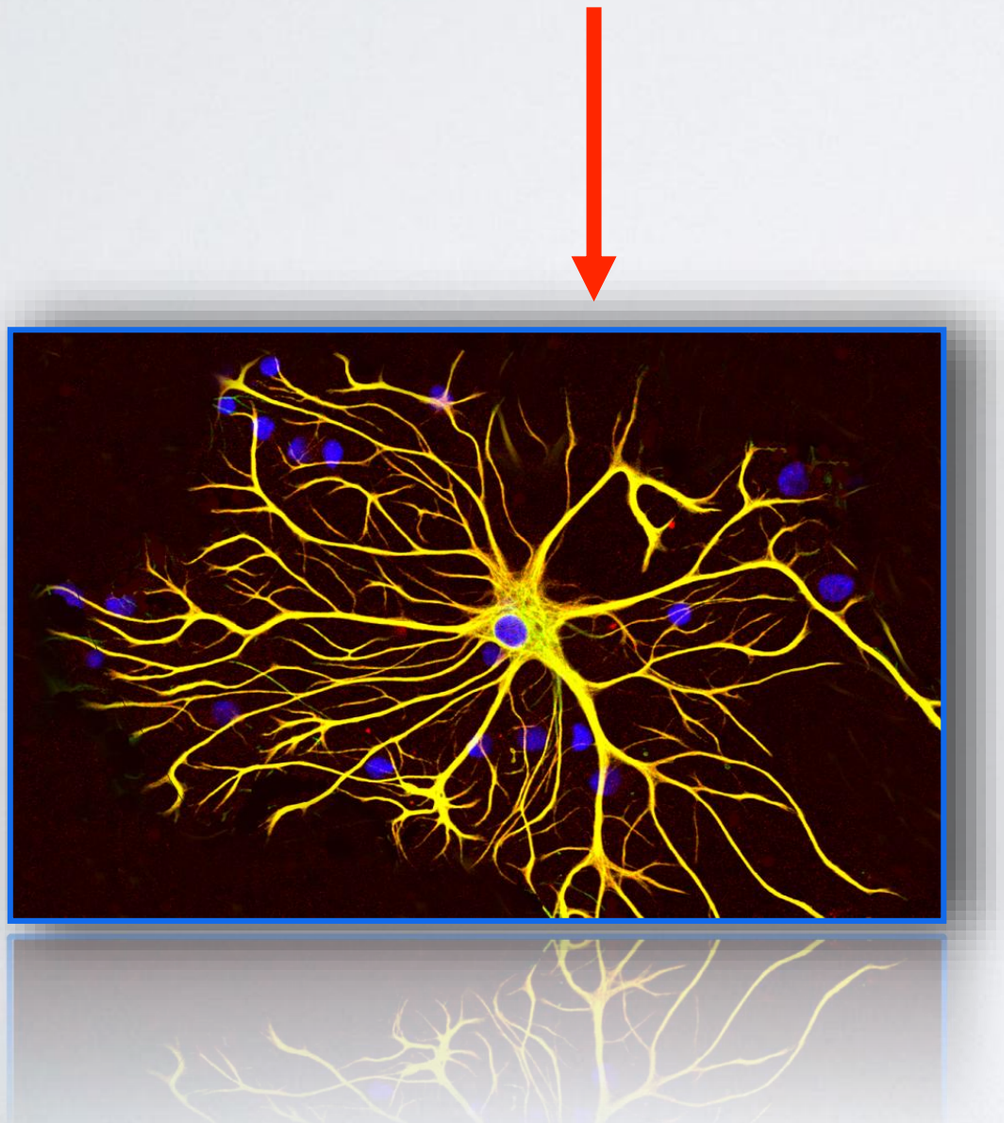
- **Oligodendrocytes**:
  - Small cells that contain many organelles in its cell body
  - Produce myelin – surrounds the axon, protects and insulates it; known as myelin sheath

---

- **Astrocytes** help neurons perform their function
  - Provide structural support for neurons
  - Aid in repair of neurons following brain damage

Understanding **Glioblastoma**

Glioblastoma is a type of astrocytoma, which is a cancer that forms in the astrocytes in the brain.

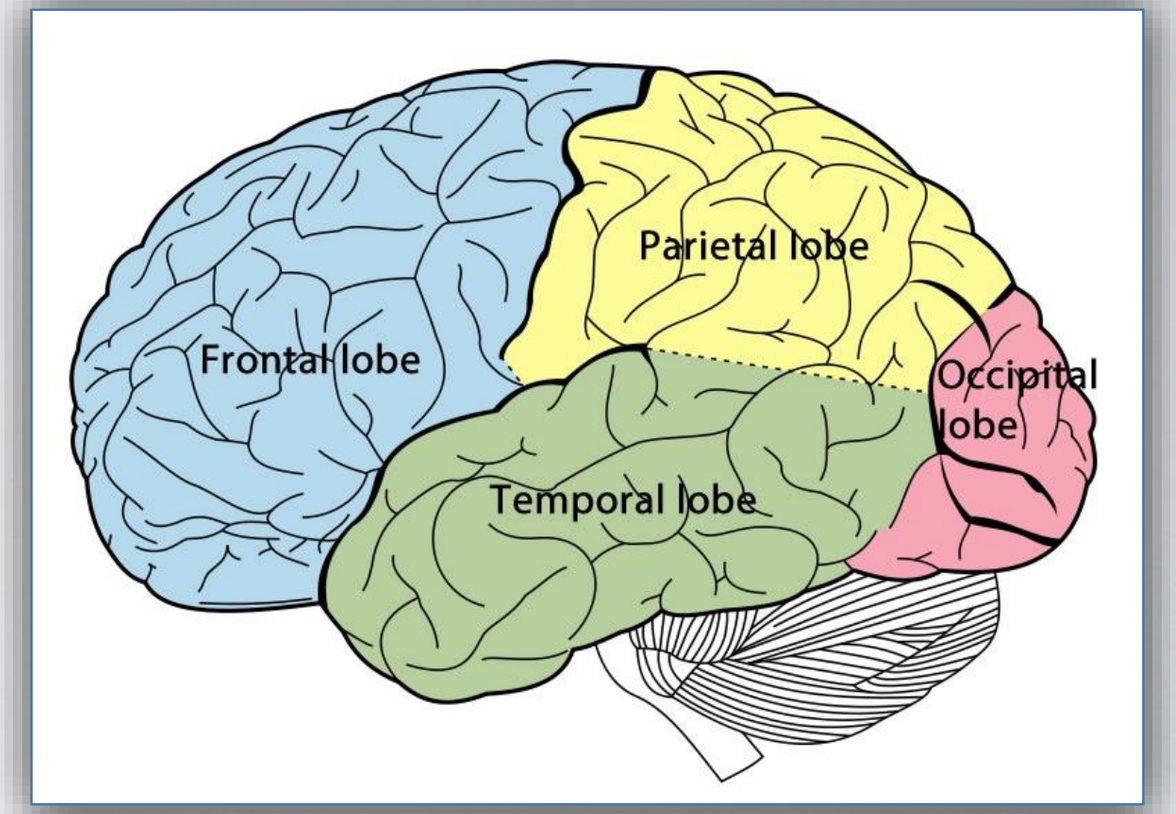


Astrocytes are the star-like cells that make up the supportive tissue of the brain.

Where is Glioblastoma **Found**?

- It is usually found in the cerebrum, which includes the frontal and temporal lobes, but could also be found in any part of the brain, and even in the spinal cord.

- Glioblastoma is a type of cancer that is very aggressive and spreads to other parts of the brain quickly.



Types of Glioblastoma

Glioblastomas usually contain a mix of cell types. These tumors contain cystic minerals, calcium deposits, blood vessels, T cells, and white blood cells.

Two Types of Glioblastoma:

1. **PRIMARY**
   Very aggressive. Form tumors quickly, and is more common.

2. **SECONDARY**
   Aggressive, but takes longer to form.
   Represents about 10% of glioblastomas.

How Common is Glioblastoma?

Glioblastoma Statistics	
Percent of all cancers	2%
Number of deaths from malignant brain tumors	2%
Percentage of fatal malignant brain tumors in males in US	55%
Age of incidence (years)	Between 60 and 84

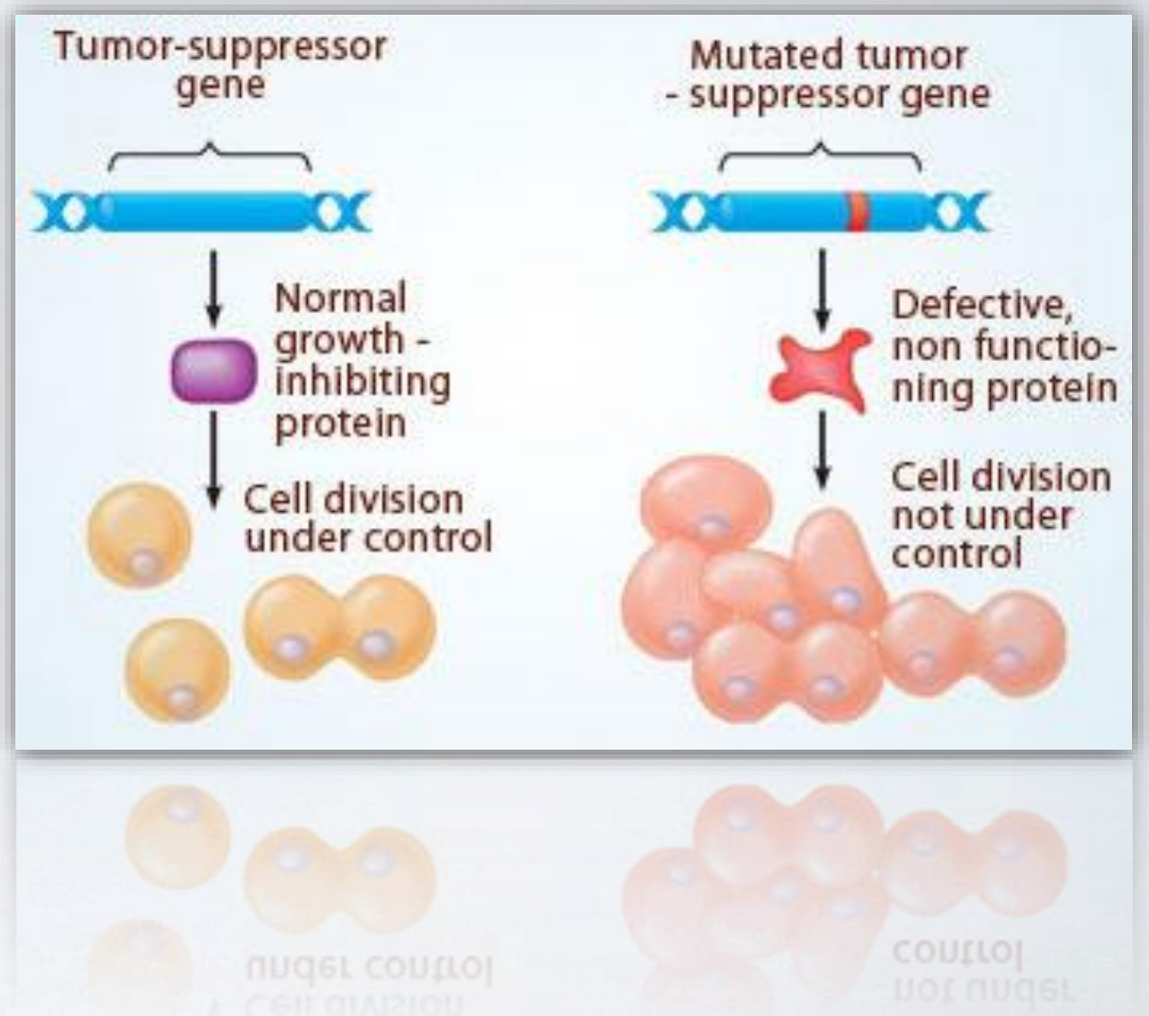
Control of Cancer

- Cancer is essentially uncontrolled cell growth
- There is a failure over the control of the cell cycle
- Two genes contribute to failure of cell cycle when mutated:

1. **Tumor-suppressor genes**
2. **Proto-oncogenes**

Tumor-suppressor gene

- Gene that makes tumor-suppressor proteins
- Prevent cell division
- Absence can lead to cancer
- TP53 is a gene that codes for making a protein called tumor protein **p53**

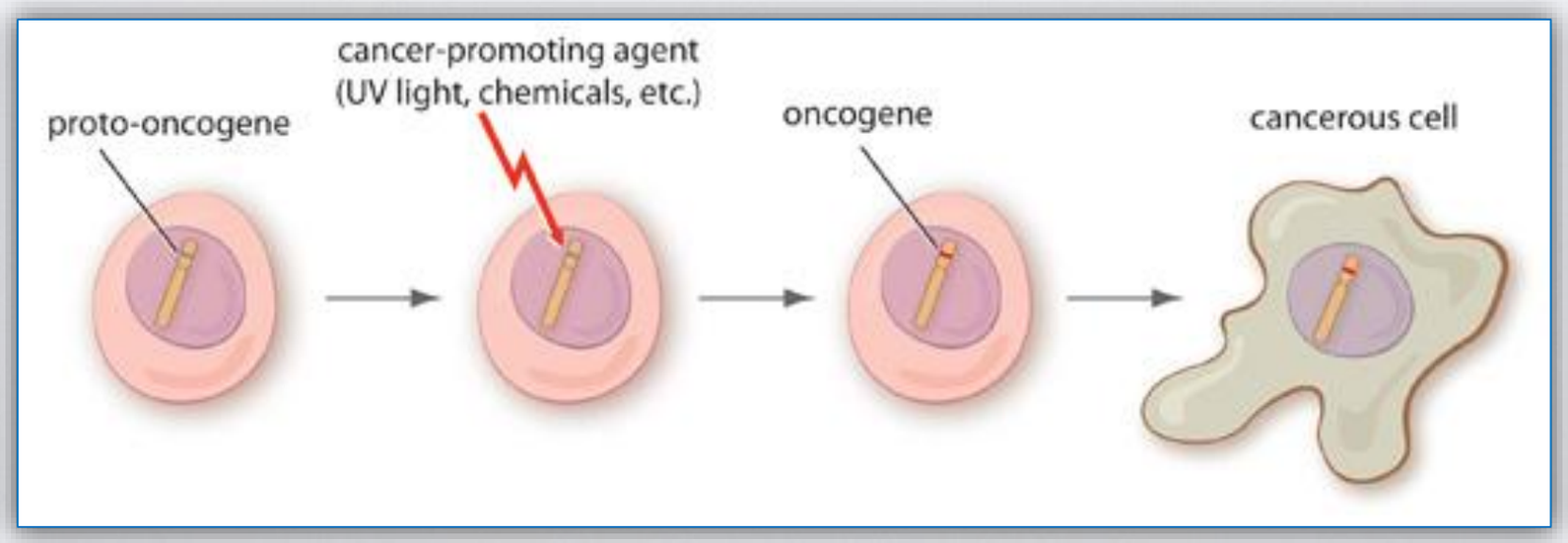


p53

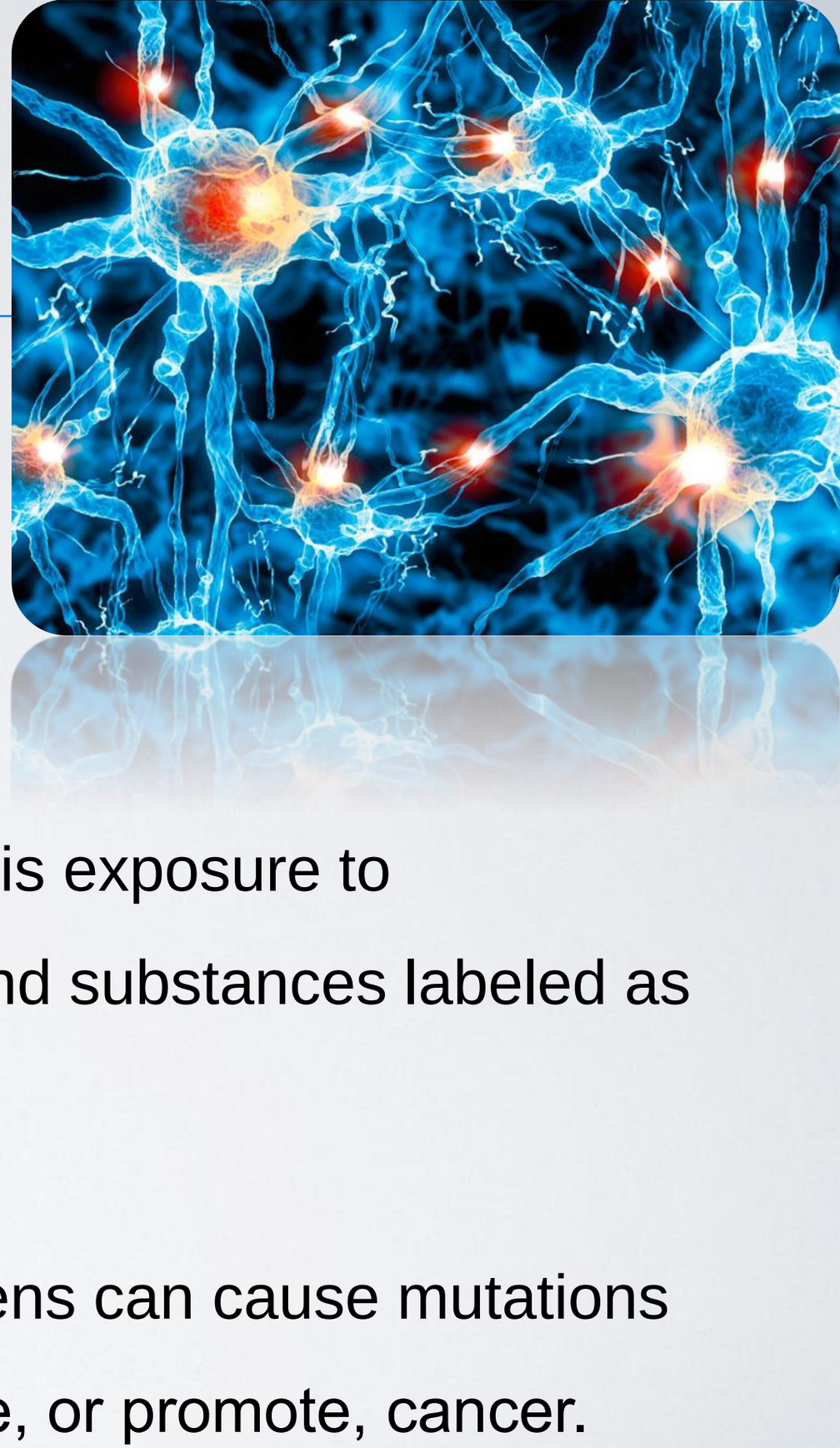
- Acts as a tumor suppressor
- Located in nucleus attached to DNA
- When DNA is damaged:
  - If it can be repaired → p53 activates genes
  - If it cannot be repaired → p53 prevents cell division and initiates apoptosis
- If p53 is damaged or absent, cell can become cancerous

Proto-oncogene

- Gene involved in normal cell growth
- When mutated, it becomes an oncogene (causing cancer)
- Oncogenes are permanently turned “on” (activated)
- Uncontrolled, rapid cell growth

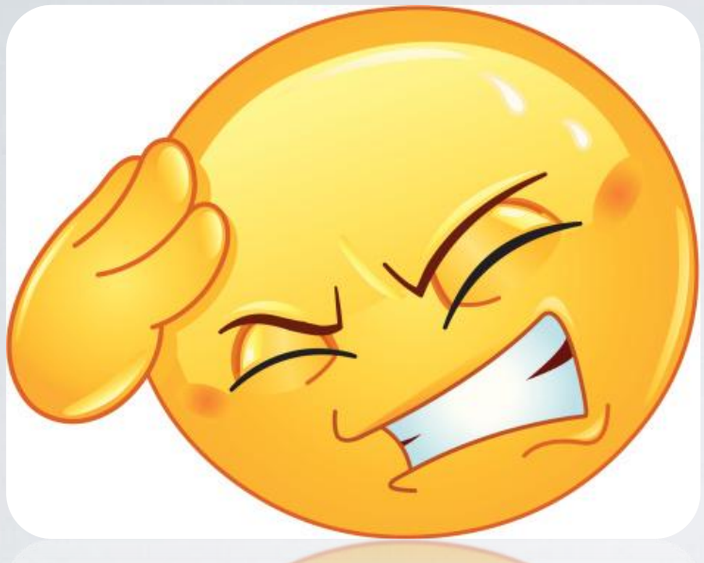


Causes



One of the known causes of brain tumors is exposure to environmental factors such as radiation and substances labeled as carcinogens.

Since exposure to radiation and carcinogens can cause mutations in a cell’s DNA, they are believed to cause, or promote, cancer.



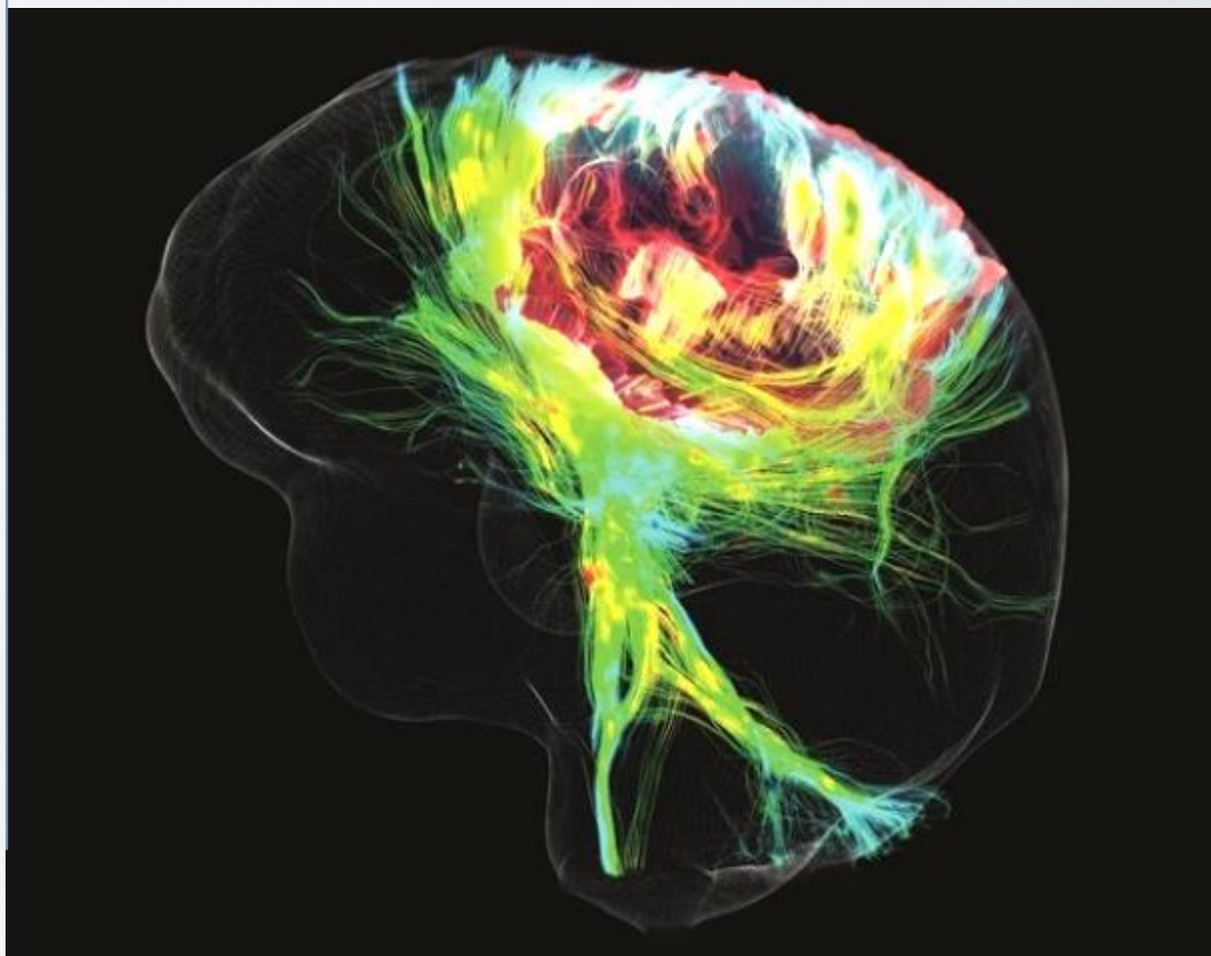
Symptoms

- Vary depending on where the tumors are located in the brain.
- The tumors cause pressure in the brain which can result in the following:
  - Headaches, nausea, vomiting, & drowsiness
  - Weakness on one side of the body
  - Memory loss
  - Speech problems
  - Visual problems
  - Seizures
  - Some symptoms are silent, and only show on a brain scan

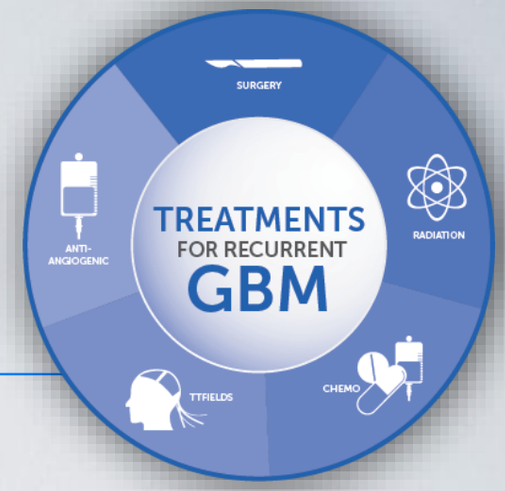
Diagnosing Glioblastoma

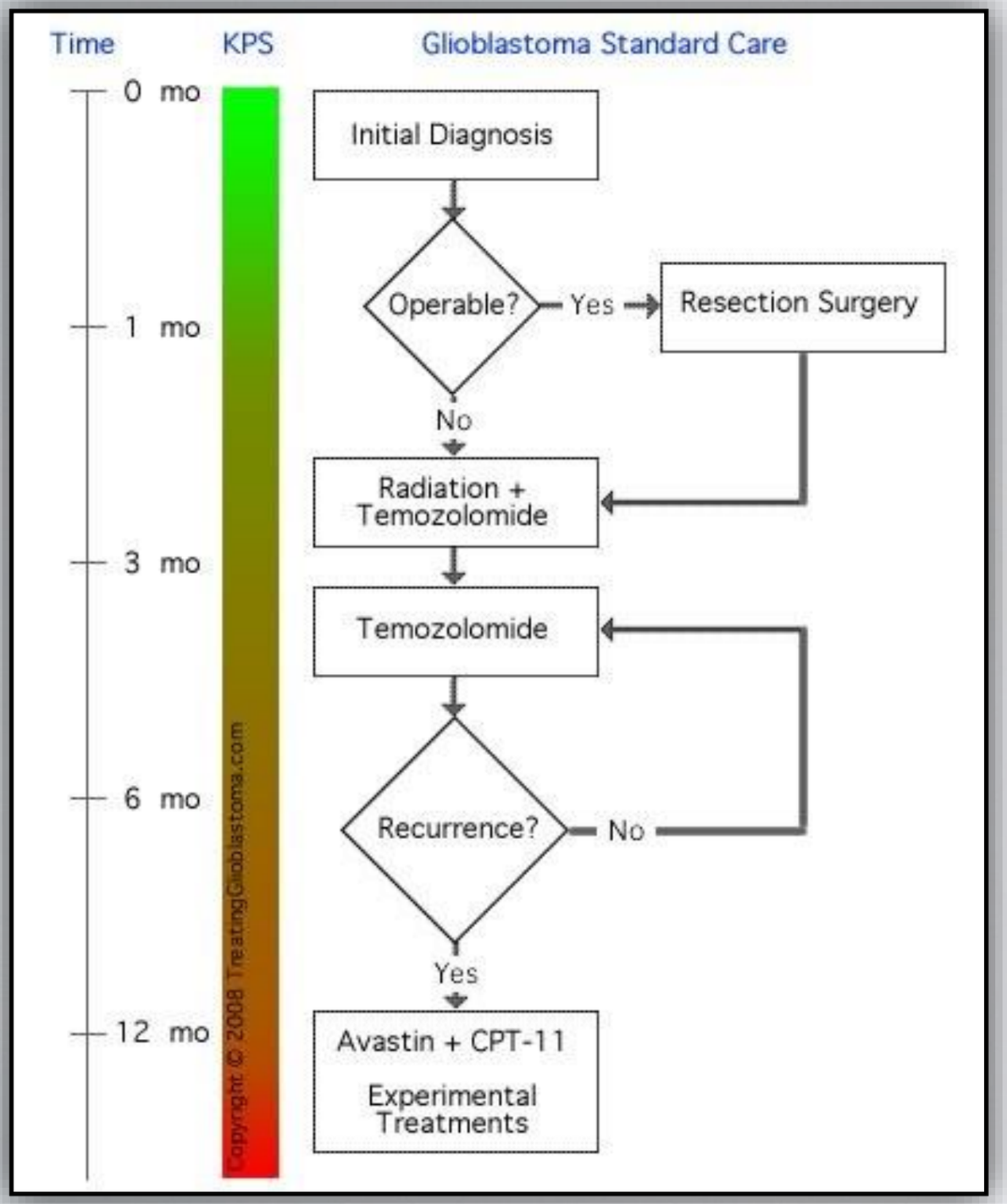
- Diagnosis involve patients undergoing CT scans and then an MRI.

- The pathological diagnosis can only be made while in surgery, when the tissue is removed for examination by a neuropathologist.



Treatment





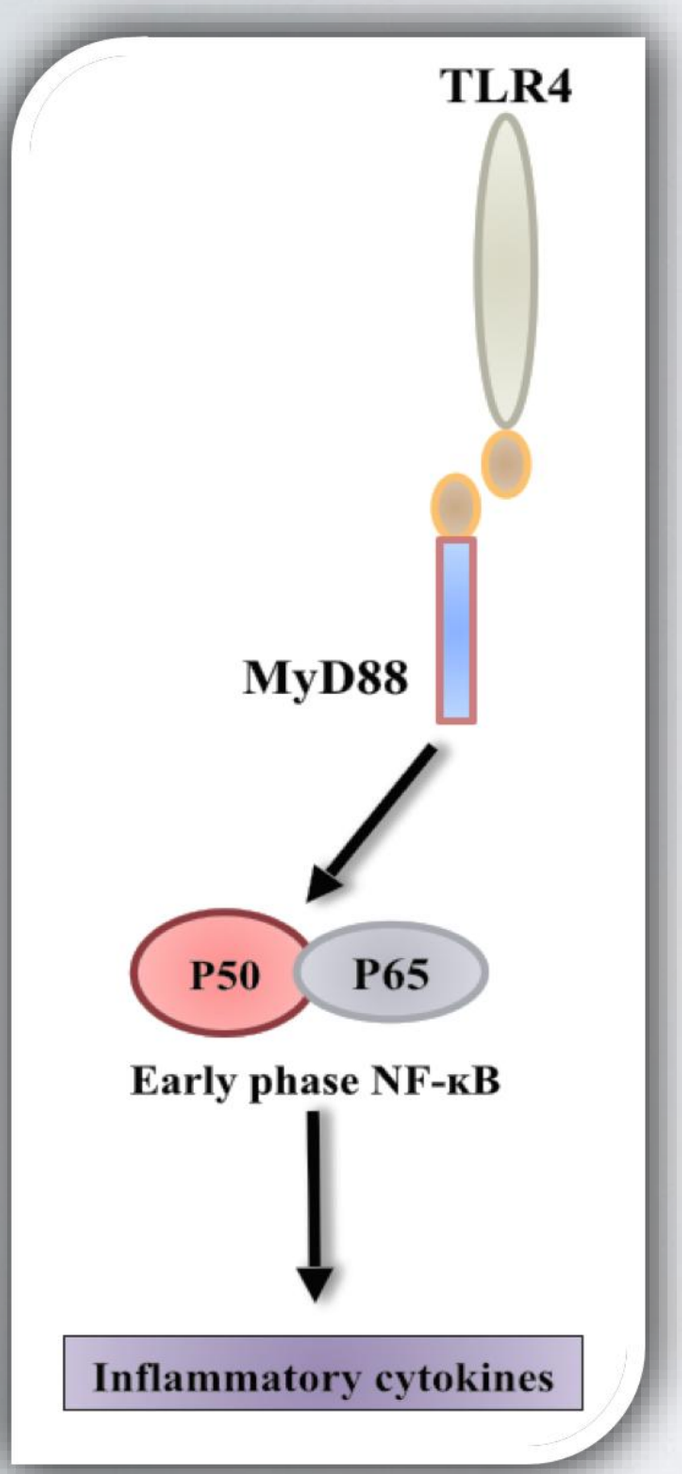
- Removal of contaminated tissue during surgery. Not all cancer can be removed due to the tumor's finger-like tentacles.

- Radiation, chemotherapy, and oral chemotherapy (temozolomide), may be used to slow down the growth of the cancer that could not be removed during surgery.

Prognosis

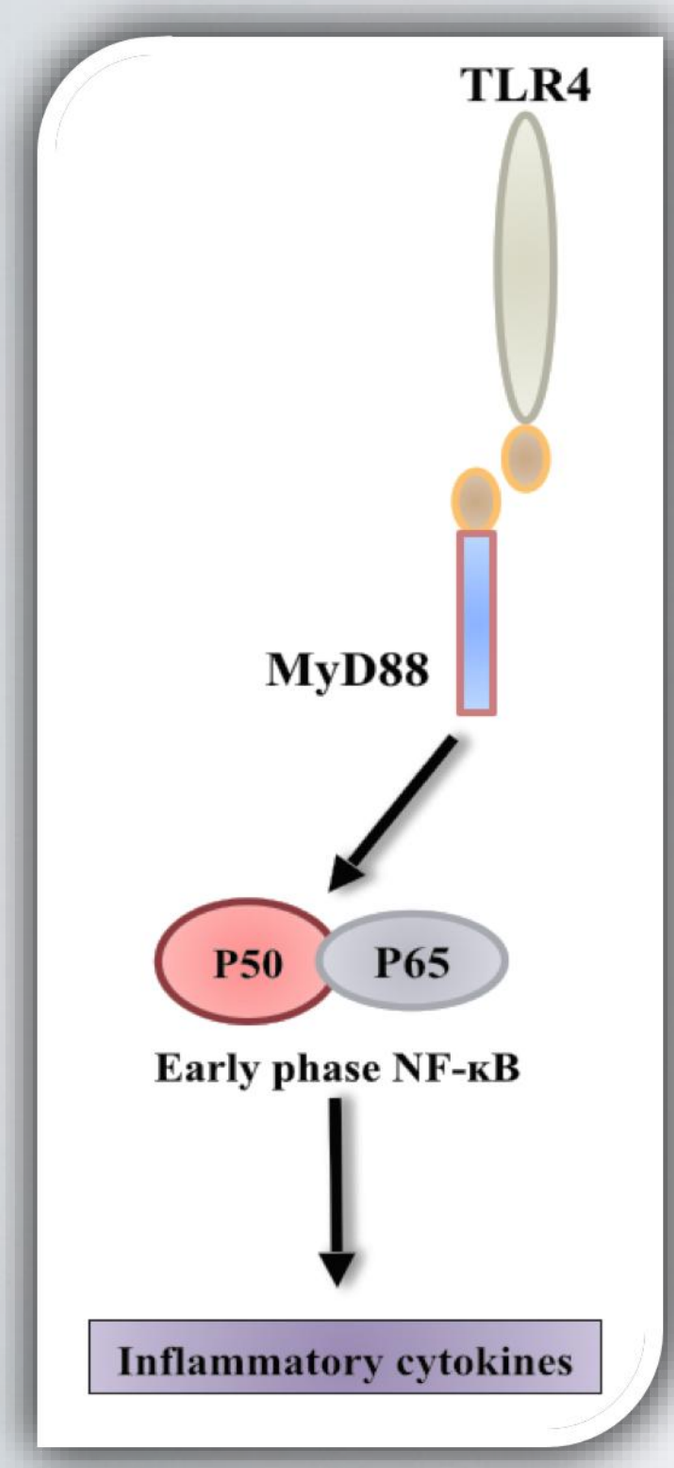
- Usually reported in years of “median survival”, which is the time that an equal number of patients do better and an equal number of patients do worse.
- Survival median for patients with more aggressive glioblastoma who have been treated with temozolomide and radiation therapy is about 14.6 months, and two year survival is about 30%.
- In 2009, however, there was a reported study with almost 10% of patients with glioblastoma living for 5 years or longer.
- Children with high-grade tumors (III and IV) usually live longer than adults; five-year survival is around 25% for children.

What is **TLR4**?



- TLR4 is a member of the toll-like **receptor protein** family which is mainly studied in immune cells.
- Patients with head and neck, esophageal, gastric, colorectal, liver, pancreatic, skin, ovarian, cervical, and breast cancer express TLR4.
- TLR4's main role is to sense foreign material and evoke an inflammatory response to protect the organism from further spreading the disease.

What is **MyD88**?



- MyD88 is an **adapter protein** used in a TLR4 signaling pathway.

- MyD88 transfers signals from TLR4 receptors, which are important for an early immune response to foreign invaders.

- TLR4 recruits MyD88 to initiate pro-inflammatory signaling.

- MyD88 produces *cytokines* (small cell signaling proteins that aid cell to cell communication).

Role of TLR4 and MyD88 in **Malignant Tumors**

TLR4 is involved in **tumorigenesis** (formation of cancer).

- **Promote antitumor immunity**
- **Increased tumor growth**
- **Immunosuppression** (complete/partial reduction of the immune response)
- **Synthesize soluble immune mediators that could help the tumor to withstand the immune attack**

Previous Research

Previous Middlesex County College students investigated the interface between the extracellular matrix and macrophages in a tumor microenvironment.

They performed in vitro assays and immunohistochemistry of patient samples and found that inflammatory cytokines (TNF-a) are present in glioblastoma, which challenged existing models in tumor biology.

Research Changes Focus

Although previous research at J.F.K. Medical Center Neuroscience Institute was focused on TNF-a, the focus of my research was centered on finding the presence of TLR4 and MyD88 in tissue samples.

TLR4 in **Glioblastoma**

TLR4 and adaptor protein MyD88 have only recently been detected in glioblastoma.

We detected them in glioblastoma using immunohistochemistry technology.

Hypothesis

Since **TLR4** and **MyD88** are expressed in other cancers, such as colorectal and breast cancer, then it should be found in glioblastoma.

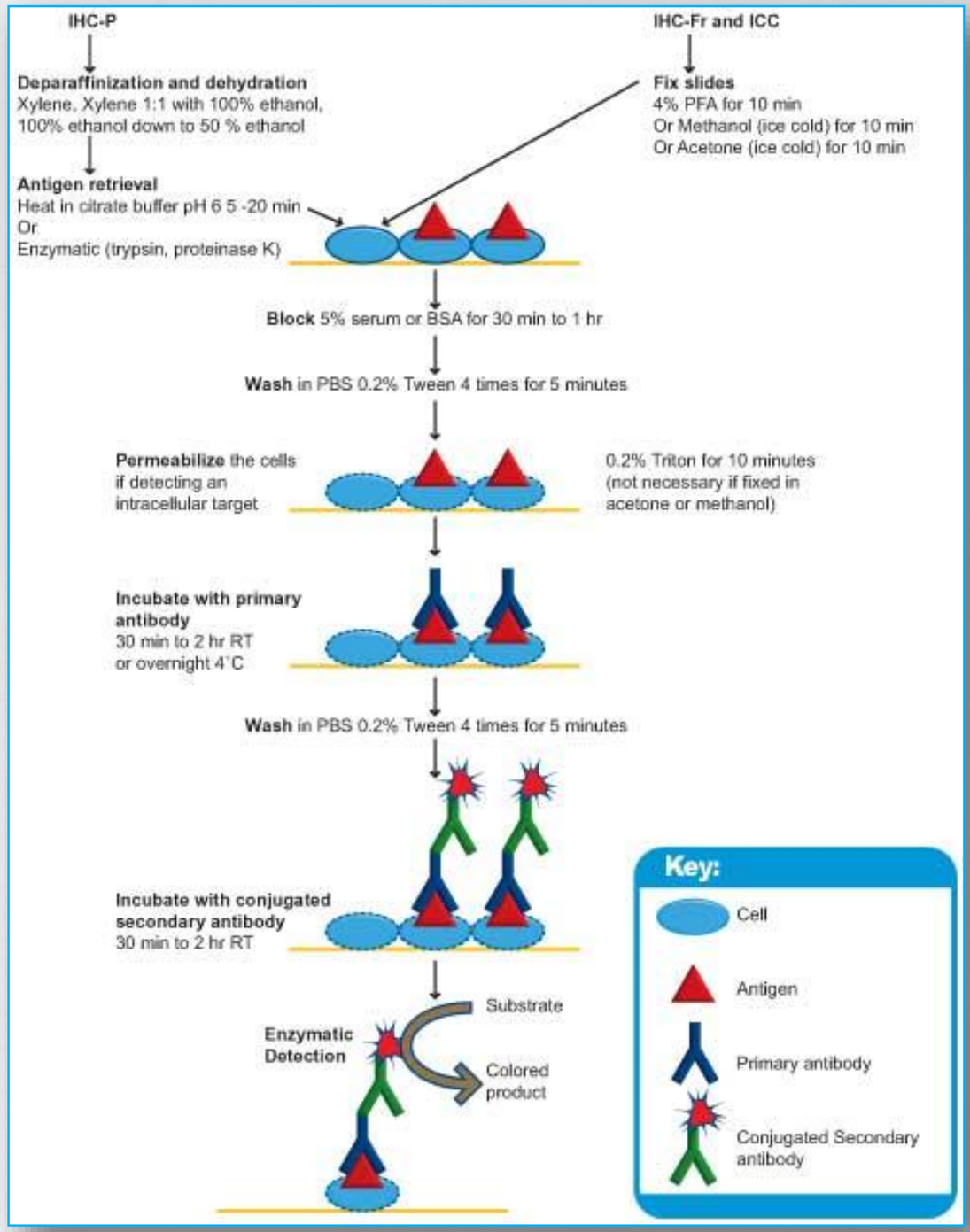
Immunohisto**chemistry** (IHC)

- A laboratory test that uses antibodies to test for certain antigens in a sample of tissue.
- It is used to diagnose different types of cancer.

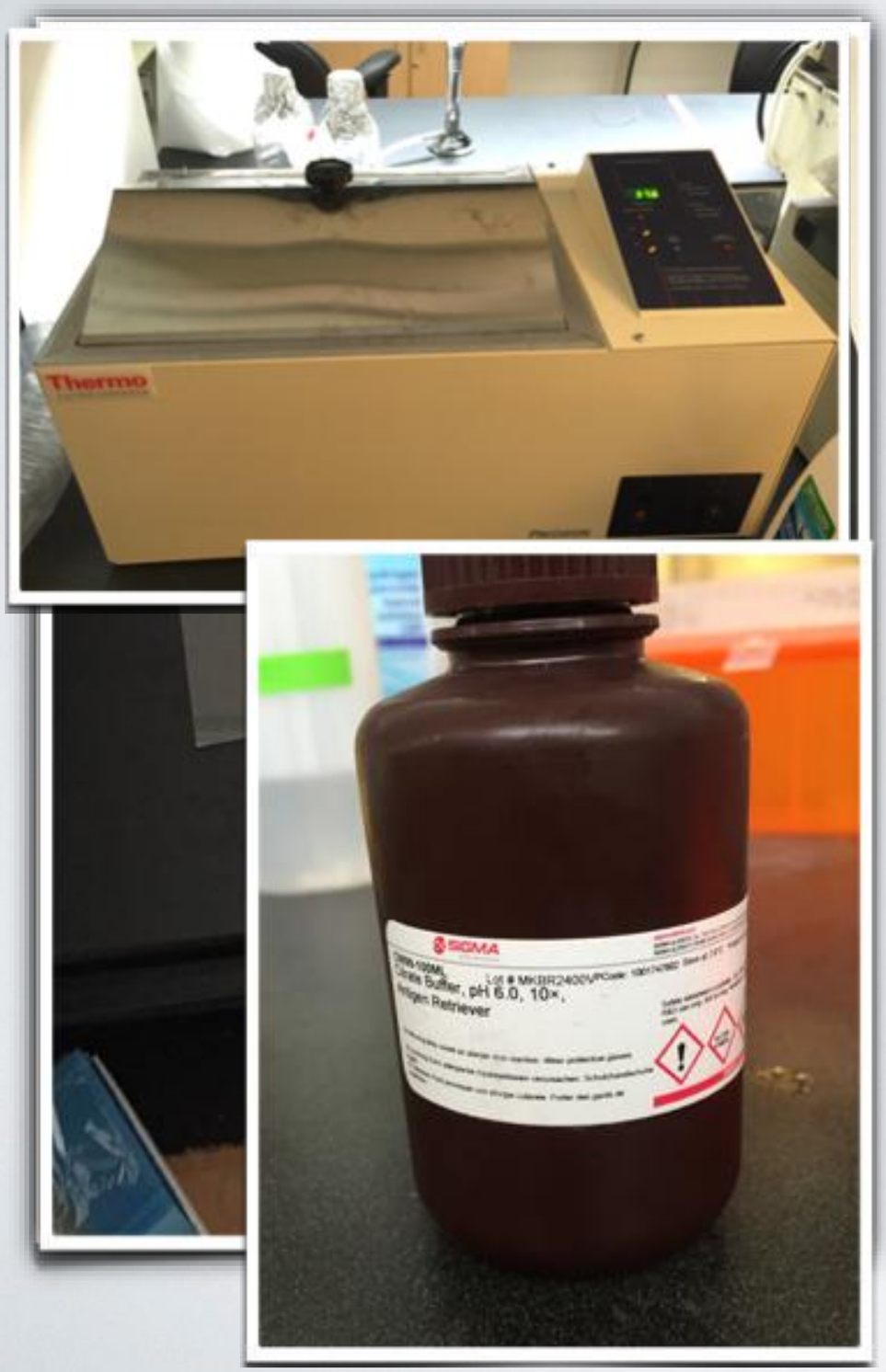
How IHC Was Used

- Used the TLR4 antibody, as well as the MyD88 antibody, to detect respective antigens.

- If TLR4 and MyD88 were expressed in the tissue, then the antigens would precipitate a brown color.



Methods: The Experiment



- Archived brain samples from the last twenty years were taken from patients.
- This included brain tumor and non-brain tumor tissue.
- Two slices were taken from each sample in order to test for **TLR4** and **MyD88**. Tumor slices were put onto slides.
- Tissues were processed.

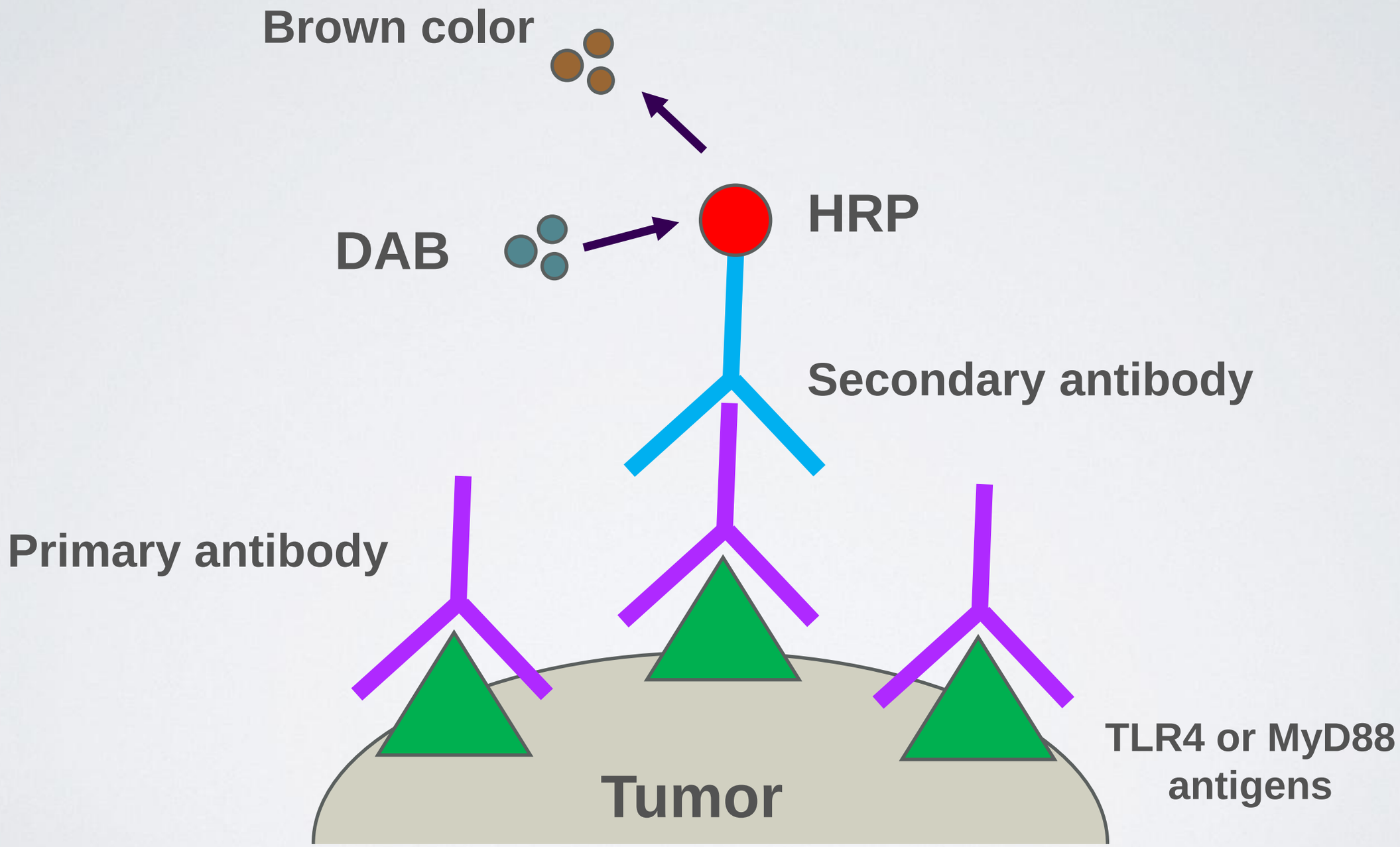
Protocol Steps

- Slice tissues and put them on slides
- Rinse them in different alcohols
- Rinse them with deionized water
- Boil them in sodium citrate
- Rinse them with phosphate buffer saline (PBS) solution
- Soaked them in hydrogen peroxide solution
- Sat in PBS solution overnight
- Rinse them again with PBS solution next day
- Put one drop of Dako HRP polymer
- Rinse with PBS solution again
- Added DAB solution
- Rinse with deionized water and stain with hematoxylin
- Rinse with ammonium hydroxide
- Rinse with deionized water and then several alcohols
- Placed cover slide using Permount

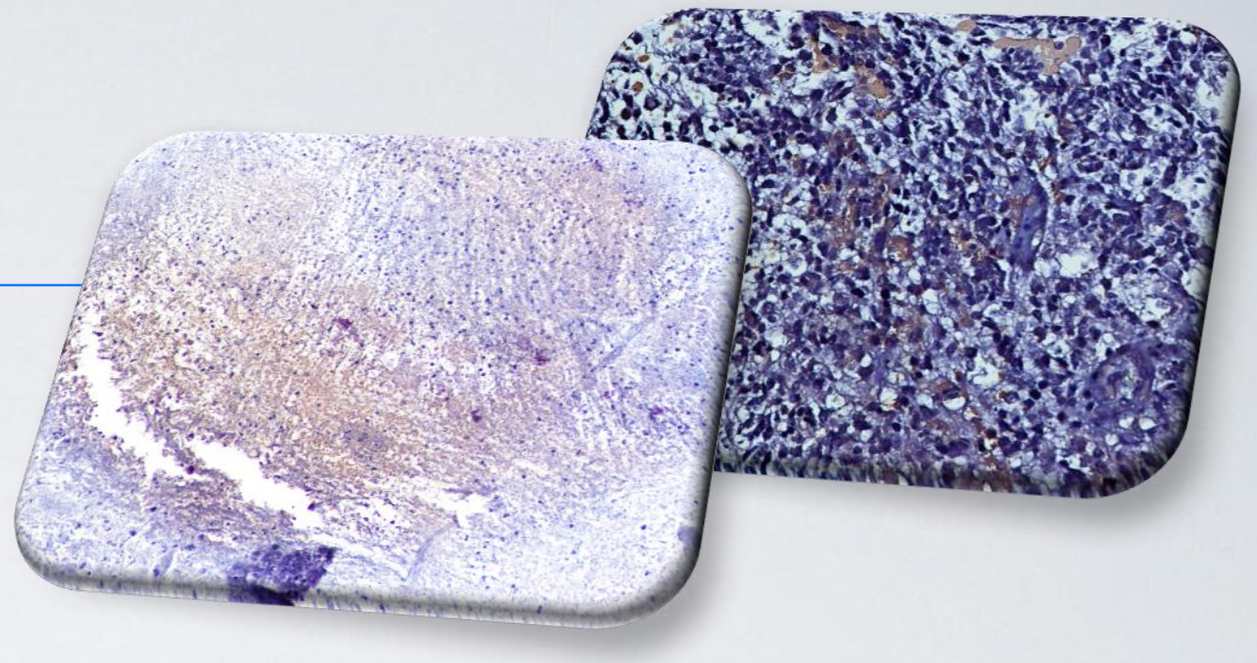
Methods: The Experiment

- Archived brain samples from the last twenty years were taken from patients.
- This included brain tumor and non-brain tumor tissue.
- Two slices were taken from each sample in order to test for **TLR4** and **MyD88**. Tumor slices were put onto slides.
- Tissues were processed.
- Examined finished slides under microscope.

IHC: A Closer Look



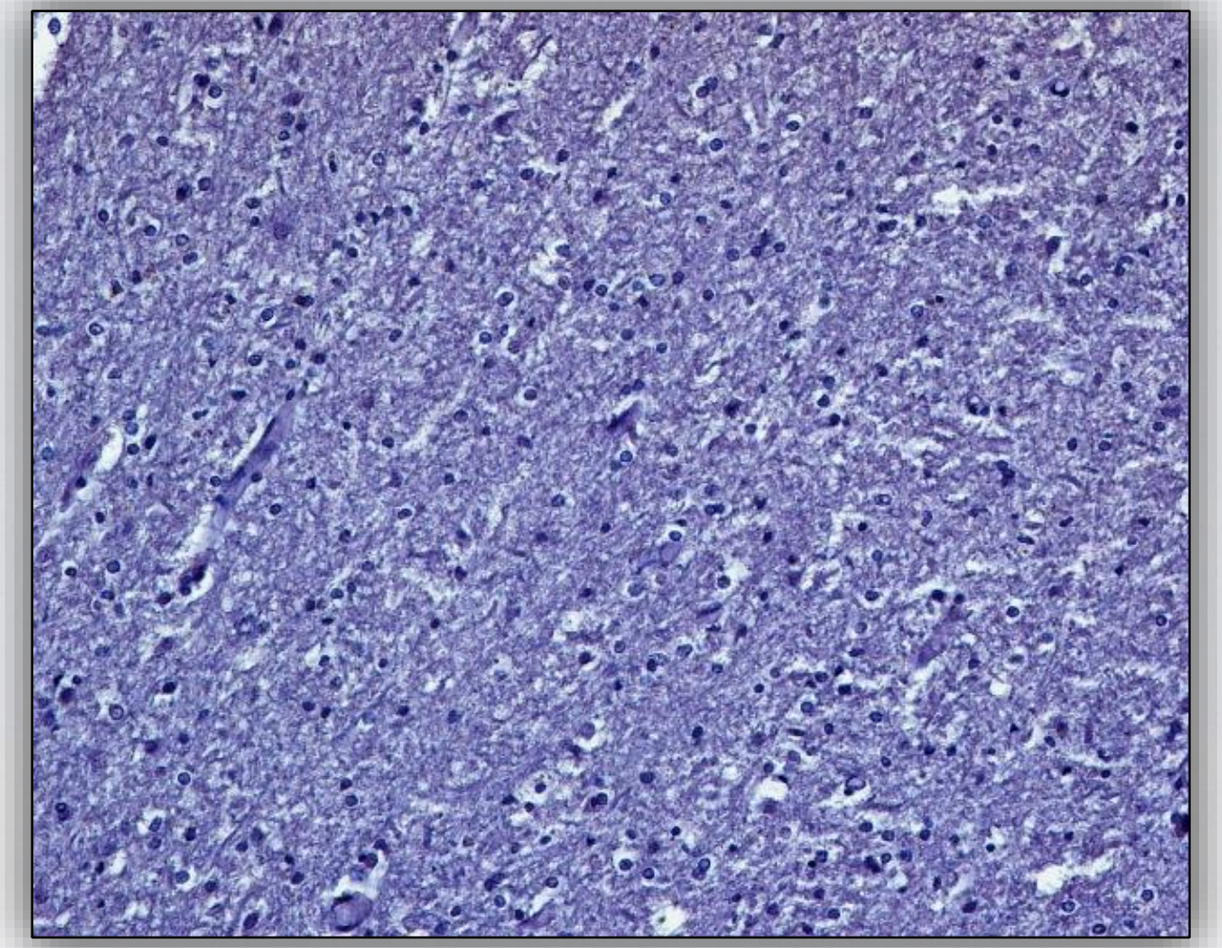
Results



- **12** out of the **12** patients had **positive** results for TLR4 and MyD88 antigen.

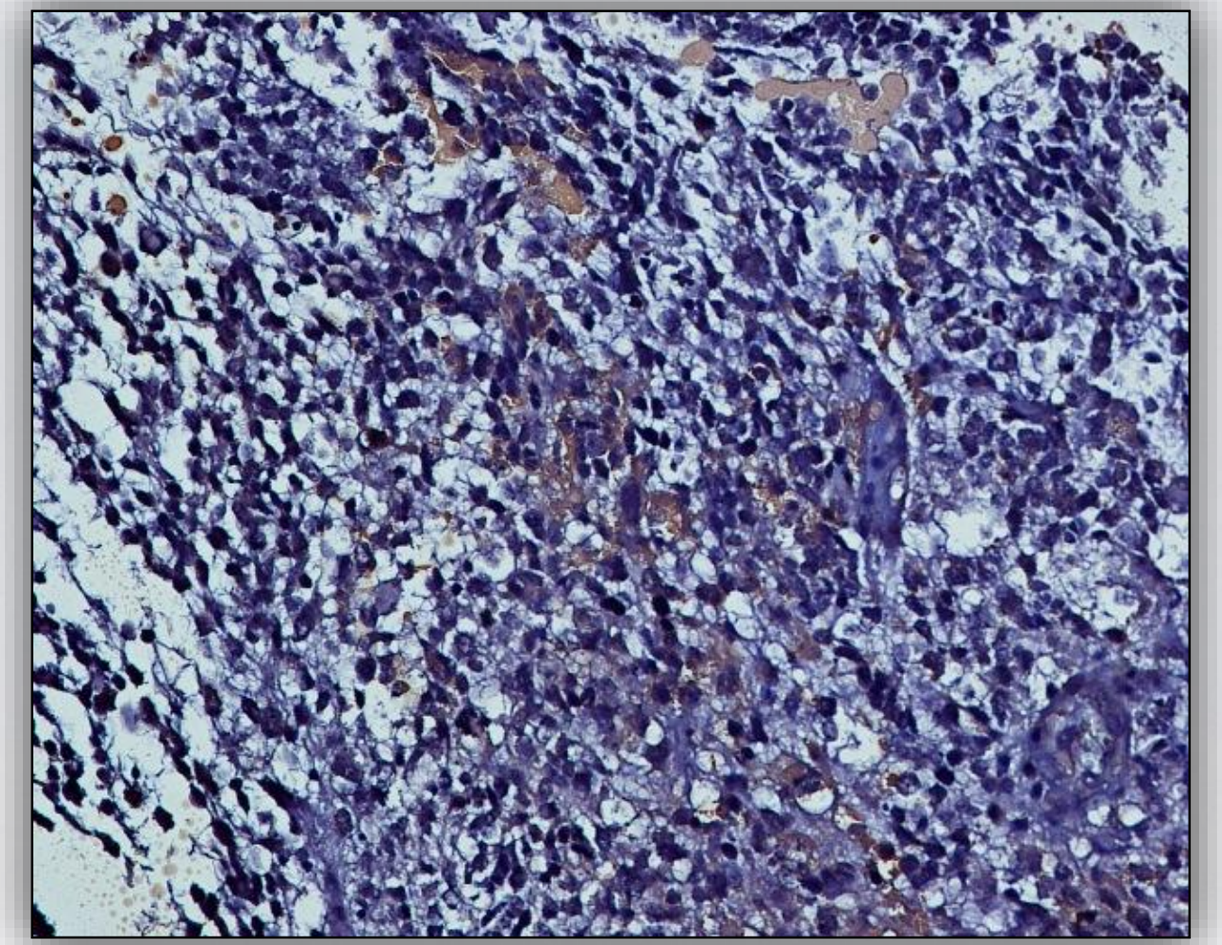
- **ALL** slides (except for the negative control) turned brown in areas where **TLR4** and **MyD88** were expressed.

Negative Control – Normal Brain



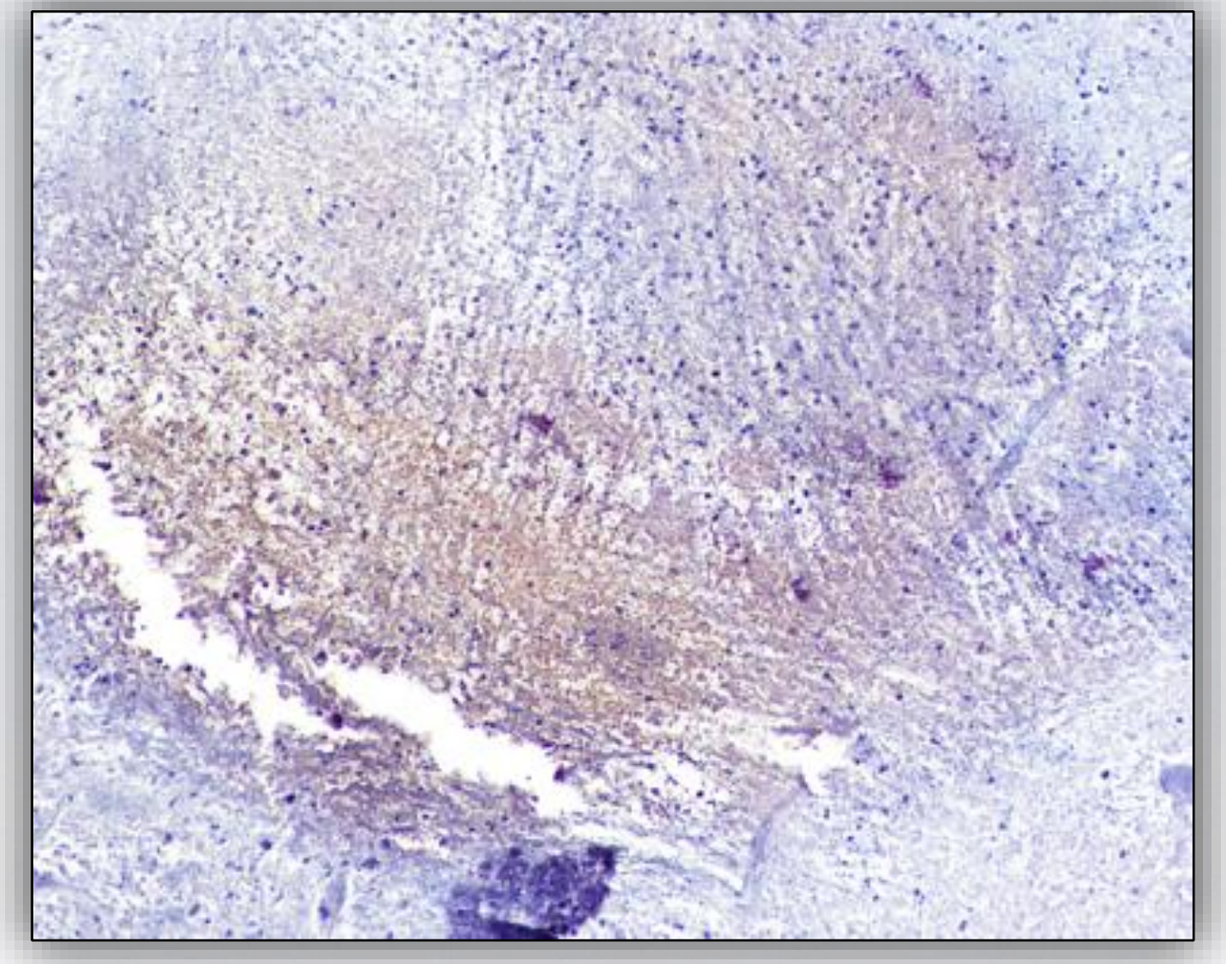
100 um

TLR4



50 um

MyD88



100 um

CONCLUSIONS

TLR4 (*receptor protein*) and **MyD88** (*adapter protein*) are expressed in glioblastoma tumors. This suggests that TLR4 and MyD88 play a role in tumorigenesis.

Possible roles include:

- Proliferation
- Cell survival from chemotherapy
- Immunosuppression

FUTURE Research

Future experiments will use glioblastoma cell lines to determine the role of TLR4 and MyD88 in tumorigenesis.

Acknowledgments

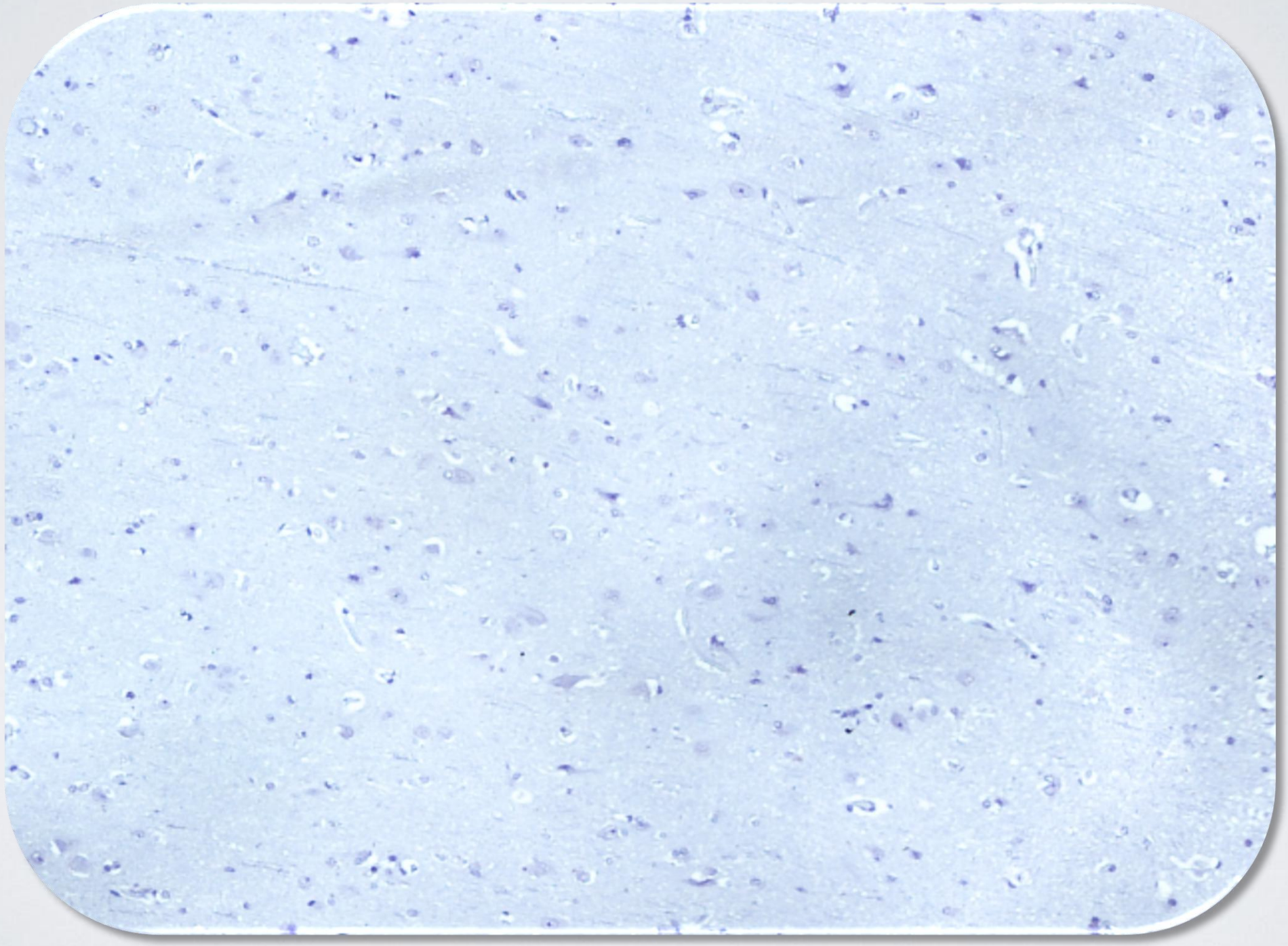
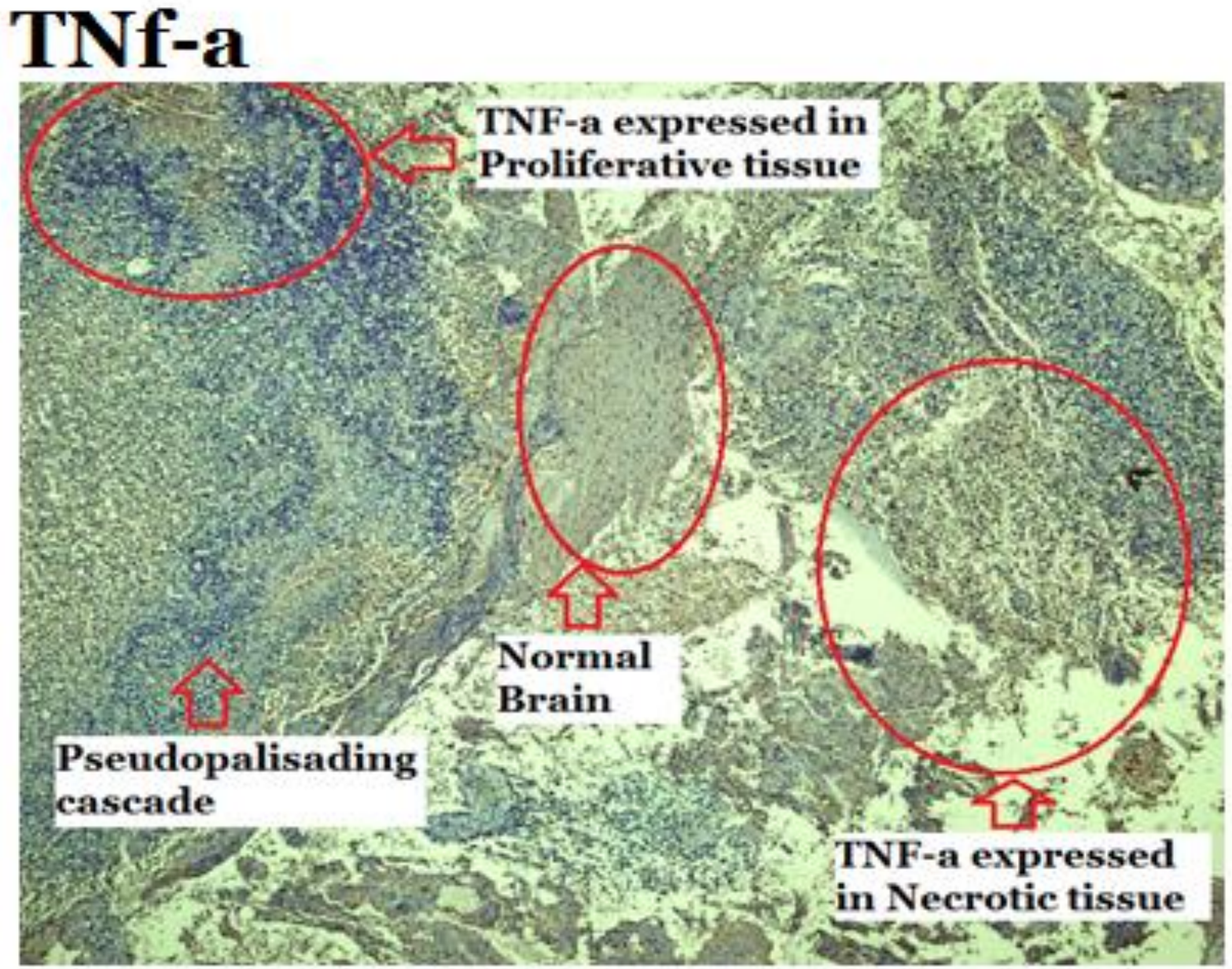
- Dr. Haim Baruh for the NJ Space Grant NASA Fellowship.
- Mentor Michelle Previtera, PhD; J. Landofi, DO; S. Bloomfield, MD; C.A. Ewing, MD and the JFK Neuroscience Institute.
- Dr. Parag Muley and Middlesex County College.
- Professor Virender Kanwal for this amazing research opportunity.
- Carmo Ferreira for assistance and support.

Thank You!

Demographics

Male:Female	**8:4**
Age Range (years)	**48-73**
Age Mean (years)	**63**
Patients with Glioblastoma Multiforme	**12/12**

Negative Control – Normal Brain



Positive Control