Morphological and Immunocytochemical Investigation of Canine Oligodendrogliomas

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(Abstract)

Previous studies of human oligodendroglial neoplasms have demonstrated the diagnostic and prognostic values of histomorphologic features and immunocytochemical markers. Primary spontaneous canine intracranial tumors share many of the biologic behaviors and pathologic features of their human counterparts. The objectives of this study were to determine if associations existed between five histomorphologic features (mitoses, cellular atypia, necrosis, vascular hypertrophy, and vascular proliferation), and three immunocytochemical markers (GFAP, EGFR, and Ki-67 labeling index) and the degree of malignancy, as defined by WHO grading criteria, of 15 canine oligodendroglial tumors. Of the histomorphologic variables examined, mitoses and cellular atypia were significantly greater in Grade III oligodendrogliomas than in Grade II oligodendrogliomas ($p = 0.002$, and $p = 0.004$, respectively), but no differences were noted between these features and Grade II oligoastrocytomas and Grade II or Grade III oligodendrogliomas. No significant associations were found between GFAP or EGFR immunoreactivity and tumor type or grade. The median percentage of Ki-67 immunoreactivity was significantly different between all tumor types and grades ($p < 0.05$), and was significantly higher in Grade III oligodendrogliomas than in both oligoastrocytomas ($p = 0.014$) and Grade II oligodendrogliomas ($p = 0.006$). Results of this study indicate that although mitoses and cellular atypia are useful histomorphologic features for the differentiation of tumors with oligodendroglial phenotypes, none of the variables examined reliably distinguished mixed gliomas from oligodendrogliomas. The presence of GFAP immunoreactivity in all tumor types suggests that oligodendroglial tumors may arise from a common multipotential cellular lineage. Similar to what has been demonstrated in humans, the Ki-67 labeling index correlated well with the degree of malignancy in the tumors studied.

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Multimedia Object Listing

Alphabetical Key of Abbreviations

Abbreviation	Translation
CGH	Comparative genomic hybridization
CSF	Cerebrospinal fluid
CT	Computed tomography
CNS	Central nervous system
EEG	Electroencephalogram
EGFR	Epidermal growth factor receptor
EM	Electron microscopy
FISH	Fluorescence *in situ* hybridization
GFAP	Glial fibrillary acidic protein
H&E	Hematoxylin and eosin
HER	Human epidermal growth factor receptor
IF	Intermediate filaments
IL-6	Interleukin-6
LOH	Loss of heterozygosity
MAG	Myelin-associated glycoprotein
MBP	Myelin basic protein
MHC	Major histocompatibility complex
mRNA	Messenger ribonucleic acid
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NFP	Neurofilament protein
NSE	Neuron-specific enolase
PCNA	Proliferating cell nuclear antigen
PDGFRα	Platelet derived growth factor receptor alpha
PCR	Polymerase chain reaction
PLP	Proteolipid protein
RT-PCR	Real-time polymerase chain reaction

Abbreviation	**Translation**
SCTBB	Stereotactic computed tomography-guided brain biopsy
VEGF	Vascular endothelial growth factor receptor
WHO	World Health Organization

Chapter 1 –Literature Review

A. Significance of comparative neuro-oncology research

Animals with spontaneously developing neoplasia provide an excellent resource for studying many aspects of cancer. Spontaneous neoplasms may behave quite differently than induced or transplanted cancers. Due to increased genetic diversity, dogs are a better comparative model than many inbred strains of rats and mice. Neoplasia of the nervous system is relatively uncommon in most domestic animals with the exception of the dog. Sixty to eighty percent of all nervous system tumors were recognized in this species and over 70% of primary central nervous system (CNS) tumors occur in dogs greater than 6 years.[1] The incidence of CNS tumors in dogs is comparable to that in the adult human population, accounting for 1-3% of all deaths in aged dogs where necropsy is performed.[1] Evidence suggests that certain breeds of dogs are predisposed to the development of specific brain tumors which strongly suggests that genetic mechanisms contribute to CNS tumorigenesis. Dogs also share the same environment as their owners and thereby may serve as epidemiologic sentinels for brain tumor development in people. Also, since the lifespan of dogs is comparatively shorter than humans, animals with intracranial tumors can be more readily studied for such factors as rate of metastasis and estimation of survival times.

Historically, animals diagnosed antemortem with gliomas have limited therapeutic options. At the time of diagnosis, animals often have developed significant clinical neurologic dysfunction as a result of the tumor or its secondary effects, and the tumors are typically large, poorly-defined, infiltrative, and intra-axial in location within the CNS. These factors ultimately prohibit complete surgical resection in veterinary medicine. Gliomas frequently demonstrate a malignant phenotype and combined with secondary tumor effects including infiltration, compression, edema and hemorrhage are often associated with rapid clinical deterioration and death. Similarities between animal and human physiology and metabolism also permit comparisons of different treatment modalities such as surgery, radiation and chemotherapy to be made. Finally, since owners frequently permit necropsy examination, crucial information regarding local tumor control and occult metastasis can also be gathered. This may ultimately not only

impact dogs afflicted with CNS tumors, but also offer insight in the pathogenesis, biologic behavior, and treatment of comparable human tumors.

B. Neuroglial structure and function

In 1846, a non-neural and interstitial substance composed of spindle-shaped cells was identified in the CNS by Virchow.[2] These cells were morphologically different from neurons and subsequently named neuroglia. In the early twentieth century, further classification of this substance divided the cellular components into distinct types including true glial cells, or macroglia, and microglia. These cell types share a close interrelationship with neurons, but they differ in many aspects. Glial cells do not generate or conduct action potentials, contain only extended processes rather than dendrites or axons, possess no synaptic contacts and they retain their ability to divide throughout life, in particular when responding to injury.[2,3] Initially, glia were believed to function in strictly a supportive role for neurons, but it is now known that glia have a diverse range of physiological roles in the normal brain and most react in a protective fashion to varying forms of injury.[4]

Embryologically, macroglial cells share a common origin and all originate from neuroectoderm. These cells are characterized into the several distinct subclasses including oligodendrocytes, astrocytes, NG-2 glia, and ependymal cells.

Microglia

Microglial cells have an embryological origin different from that of macroglial cells in that they are derived from mesodermal tissue.[3] These cells have spindle-shaped bodies and a thin rim of densely staining cytoplasm which is difficult to distinguish from the nucleus. In a number of disease states, such as trauma, microglia are stimulated and migrate to the area of injury. During this time, they are capable of phagocytosis of degenerated cells and debris. There is strong evidence that microglia express class II MHC upon activation, frequently in the absence of a T-cell response.[4] Microglia also produce numerous proinflammatory cytokines including IL-6 and transforming growth factor-β which often indicate the first evidence of a homeostatic disturbance within the CNS.[2,4]

Macroglia

Ependymal cells

Ependymal cells form a continuous epithelium which covers the walls of the cerebral ventricles and the central canal of the spinal cord.[3] They are grouped into two main classes: ependymal cells of the choroid plexus and extrachoroidal ependymal cells. Choroidal ependymal cells provide an active barrier between the fenestrated capillaries of the choroid plexus and the cerebrospinal fluid.[3] It is at this site where CSF is produced involving ultrafiltration through the capillaries, as well as secretion by the endothelium via active transport of sodium over the choroidal epithelial wall. The extrachoroidal ependymal cells primarily function in movement of cerebrospinal fluid by use of their cilia which extend into the ventricular cavity.[2]

Provided that cerebral perfusion pressure is adequate, overall CSF production is a process which remains relatively constant. Once produced, CSF circulates slowly through the ventricular system passing from the lateral and third ventricles via the mesencepahlic aqueduct into the fourth ventricle. CSF leaves the ventricular system at the lateral foramina of the fourth ventricle into the subarachnoid space in the cerebellopontine angle. It then flows over the surface of the brain and spinal cord within this space, ultimately absorbed into the venous system by the arachnoid villi.[4]

NG-2 glia

NG-2 glia are a population of cells which express NG-2 chondroitin sulfate proteoglycan and are found in both gray and white matter. They function in response to CNS injury, specifically by upregulation of NG-2 expression, proliferation and outgrowth of glial processes.[4] NG-2 glia may also assist in the development of nodes of Ranvier and the aggregation of sodium channels.[4] These cells are phenotypically distinct from other glial cells, although it has been theorized that they may give rise to oligodendrocytes, and possibly other glial cells, in the developing and adult CNS.[4] Differentiated NG-2 glia also express similar antigens as oligodendrocytes and are upregulated in glial neoplasms, including glioblastomas, astrocytomas, and oligodendrogliomas.[5] This finding has supported the theory that these various tumors may all be derived from NG-2 glia.[5]

Astrocytes

Astrocytes are multipolar cells with numerous fine and tortuous processes. The cell body is approximately 10μm in diameter and the processes can extend out over 50μm.[3] The terminal enlargements of the astrocytic processes often come in contact with neurons or non-neuronal tissue such as blood vessels.[3]

Traditionally, astrocytes are divided into two subtypes including fibrillary, or fibrous, and protoplasmic astrocytes. Although both forms represent variations of the same cell type, it has been proposed that the two groups are derived from different progenitor cell types and that fibrous astrocytes may also originate from the same progenitor as the oligodendrocyte.[6]

Protoplasmic astrocytes are typically located within gray matter in close relation to capillaries. They range in size from 10 to 40μm and have a paler staining cytoplasm than the fibrous type. Fibrous astrocytes are located within white matter and contain larger numbers of densely-packed 9-nm glial filaments.[3] These intermediate filaments have an average diameter of 8-10μm and are composed of glial fibrillary acidic protein (GFAP), distinct from that of other neurofilaments. Historically, this characteristic has been used as a method for immunocytochemical identification of astrocytes and distinguishing them from neurons and other types of glial cells.

Astrocytes function in several different capacities. They provide nourishment and structural support for other CNS components including synaptic complexes and the bodies of some neurons, such as Purkinje cells.[2] Astrocytes also function during CNS repair. Changes observed within astrocytes during CNS repair include proliferation, swelling, glycogen accumulation and gliosis. Other astrocytic functions involve transport mechanisms (primarily of water and electrolytes), metabolism of excitatory neurotransmitters and exogenous drugs, and regulation of local pH levels. Astrocytes are also considered to be involved in the induction and possibly maintenance of the blood-brain barrier. However, at the present time, no optimal *in vitro* barrier model exists and much remains to be done to determine the exact mechanisms involved.[4]

Oligodendrocytes

Two types of oligodendrocytes have been identified including interfascicular, or myelinizing, and satellite oligodendrocytes. The interfascicular type are found in white matter where they form the sheaths of myelinated axons. Their cell bodies are small with a diameter of 6-8μm and contain few processes.[7] Satellite oligodendrocytes are smaller in diameter, restricted to gray matter and closely adhered to the surface of neurons.[4] Oligodendrocytes can be distinguished from astrocytes based on several ultratructural characteristics: the absence of glial filaments, presence of 24-nm microtubules at the margins of the cells, and presence of lamellar dense bodies, a typical feature of oligodendrocytes.[7]

Myelinization by oligodendrocytes represents a critical stage in the development of the nervous system. Once complete, one oligodendrocyte is capable of forming 20 to 70 myelin sheaths around different axons.[4] Therefore, degeneration or dysfunction of a single oligodendrocyte can ultimately cause the disappearance of myelin segments on numerous different axons.[4] Myelin is the compacted glial plasma membrane composed of 70% lipids and 30% proteins on a dry weight basis.[7] The lipids composing myelin include cholesterol, phospholipids and glycolipids. Specifically, glycolipids are found in higher concentrations within myelin as compared with other cell membranes. Two glycolipids, in particular, galactoceramide (gal-C) and sulfogalactosyl ceramide, are unique to myelin. These features assist in oligodendrocyte identification as well as aiding in myelin conduction of action potentials.[3] The proteins of myelin are less abundant compared to other cell membranes. Two types of protein are characteristic of central myelin including myelin basic proteins (MBPs) and proteolipid proteins (PLPs). MBPs are located on the cytoplasmic side and function in internal communication within the oligodendroglial plasma membrane. PLPs are integral membrane proteins and represent approximately 50% of the total myelin protein. Their function has not yet been elucidated.[7]

C. Classification of human gliomas

Tumors of the central nervous system can present with numerous histological appearances and cytological varieties. This likely only reflects the complex morphology

of the organ system from which they arise. Attempts to classify CNS tumors dates back nearly 150 years, but more recently an international collaboration defined the pathology and genetics as well as outlined the grading of each tumor type of the nervous system. The updated edition published in 2000 has become the official World Health Organization (WHO) classification scheme.[8]

Glial tumors (gliomas) include the following: astrocytomas, oligodendrogliomas, mixed gliomas, ependymal tumors, and choroid plexus tumors. Astrocytomas are subdivided into diffuse astrocytomas (WHO grade II), anaplastic astrocytomas (WHO grade III) and glioblastomas (WHO grade IV). Other less common astrocytomas include pilocytic astrocytomas (WHO grade I), pleomorphic xanthoastrocytomas, desmoplastic cerebral astrocytoma of infancy and subependymal giant cell astrocytomas. Oligodendrogliomas are subdivided into oligodendrogliomas (WHO grade II) and anaplastic oligodendrogliomas (WHO grade III). Mixed gliomas require the identification of at least two unequivocally neoplastic components resembling different macroglial lineages, i.e., astrocytic, oligodendroglial, and/or ependymal differentiation. These tumors are subdivided into oligoastrocytomas (WHO grade II) and anaplastic oligoastrocytomas (WHO grade III). Ependymal tumors include ependymomas (WHO grade II), anaplastic ependymomas (WHO grade III), myxopapillary ependymomas (WHO grade I) and subependymomas (WHO grade I). Lastly, choroid plexus tumors are subdivided into choroid plexus papillomas (WHO grade I) and choroid plexus carcinomas (WHO grade III).[8]

Oligodendroglial tumors have recently attracted much attention since many prove to be sensitive to chemotherapy, which has been linked to certain tumor-associated genetic alterations. Additionally, humans whose tumors contain these alterations have statistically prolonged survival.[9] Thus, oligodendrogliomas are unique in that they have become the first human brain tumor for which advanced immunocytochemical and molecular diagnostics have become integral for therapeutic planning and prognostication. The remainder of this review will focus on the comparative aspects of oligodendroglial tumors in humans and their canine counterparts.

D. Pathology of human oligodendrogliomas

As a group, oligodendroglial tumors include low-grade oligodendrogliomas (WHO grade II), anaplastic oligodendrogliomas (WHO grade III) and oligoastrocytomas, or mixed gliomas (WHO grades II and III). In humans, they are the third most common tumor of glial origin and historically have been recognized as intracranial tumors originating from oligodendrocytes.[10] They may be diagnosed at any age, but typically have two peak incidences: 6 to 12 years and 26 to 46 years.[8] Males appear to be slightly more affected than females with reported ratios of 2:1.[11]

Gross pathologic features

Oligodendrogliomas may develop in any location throughout the neuraxis, although they have a predilection for the subcortical white matter.[12] Low grade oligodendrogliomas (WHO grade II) grow slowly and appear as well-defined masses which may be pink to gray in color. Some tumors contain mucoid degeneration, making the tumor appear gelatinous.[13] Calcification is also a common finding notably in the tumor periphery and the adjacent neuropil.[14] Localization of oligodendrogliomas is variable although 50-65% of humans have involvement of their frontal lobe. The temporal, parietal and occipital lobes can also be affected, but in decreasing frequency when compared to the frontal lobe.[14] There may also be invasion into the leptomeninges and or spread through the corpus callosum, across the ependyma, or into deeper structures within the brainstem.[12]

Histomorpholologic features

On histologic examination, oligodendrogliomas classically exhibit moderate cellularity and the tumor cells have uniformly round nuclei with a swollen, clear cytoplasm.[12] The nuclei of tumor cells are larger compared to those of normal oligodendrocytes and have an increased chromatin density. The typical histologic appearance of oligodendrogliomas is often described as a “honeycomb” or “fried-egg” appearance.[12] Although this is an artifactual finding secondary to formalin-fixation, it is considered a useful diagnostic feature when it is observed.[12] Other features seen in oligodendrogliomas can include microcalcification, mucoid or cystic degeneration and a

dense network of branching capillaries, often considered in a “chicken-wire pattern”. This vascular pattern when present is another useful identifying feature of tumor cells.[12]

In humans, oligodendrogliomas may be misdiagnosed as other tumors, especially when other diagnostic tools such as frozen sections are utilized.[15] Certain glial and neuronal tumors including the clear cell ependymoma, central neurocytoma and dysembryoplastic neuroepithelial tumor share the presence of neoplastic cells with a round nucleus and clear cytoplasm. However, these tumors possess individual architectural and immunostaining patterns which aid in their diagnosis and distinction from the oligodendroglioma.[8,15]

Ultrastructural features

Distinguishing oligodendrogliomas from other types of tumors can also be aided by several ultrastructural features. On electron microscopy (EM), the cytoplasm of oligodendrogliomas has numerous ribosomes, microtubules, and glycogen granules. Additionally, the most characteristic ultrastructural feature of oligodendrogliomas is that they contain concentric arrays of membranes, also called membrane laminations, or scrolls. A fragment of the cytoplasm found within a scroll has been observed to contain mitochondria, lysosomes, and an abundance of glycogen granules.[16]

Astrocytes within oligodendrogliomas

Many tumors classified as oligodendrogliomas contain varying amounts of astrocytic cells. The presence of these cells has been hypothesized to represent reactive astrocytes trapped within an invasive tumor, transitional forms of oligodendroglial cells, or differentiated, neoplastic astrocytic cells. Oligodendroglial tumors may contain cells with the appearance of small gemistocytes called “minigemistocytes” or “transitional cells”. Compared to oligodendrocytes, minigemistocytes typically have more abundant cytoplasm, which is eccentric in location and positive for GFAP.[14] Another theory regarding the presence of GFAP-positive oligodendrocytes proposes that transient GFAP expression by myelin-forming glia occurs during normal development. The recognized “gliofibrillary oligodendrocyte” or “gfocs” were observed in half of all tumors evaluated

in one study, with the suggestion of the return to undifferentiated behavior by some neoplastic oligodendrocytes.[17]

Glial fibrillary acidic protein

The glial fibrillary acidic protein (GFAP) is the major protein of the glial intermediate filaments (IF). There are 5 classes of intermediate filaments which are currently recognized including: neurofilaments, present in neurons; vimentin filaments, present predominantly in mesenchymal cells; desmin filaments, present mainly in smooth, skeletal and cardiac muscle cells; keratin filaments, present in epithelial cells and cells of epithelial origin; and glial filaments found mainly in astrocytes.[18] The initial studies of GFAP originated during the investigation of multiple sclerosis (MS), and found that old MS plaques contain mainly fibrous astrocytes and an abundance of glial filament and neurofilament proteins.[19] It was later observed that GFAP shares many properties with other IF proteins, but unique epitopes allowed its use in immunohistochemistry for the diagnosis of astrocytic tumors, study of astrocyte development and gliosis, and the investigation of CNS regeneration.[19]

Numerous studies have used the myelin-free axon preparation as an abundant source of neurofilaments[20]; however Eng et. al. also observed in bovine white matter GFAP-positive cells or processes which were present in all subcellular fractions including myelin, indicating sources other than astrocytes may contain GFAP.[18] Other reports have also described tumors containing GFAP-positive oligodendroglial neoplastic cells, further supporting GFAP immunoreactivity in all types of gliomas, including limited numbers of oligodendrogliomas.[21,22]

E. Grading of human oligodendrogliomas

Controversy still exists regarding the diagnosis, classification and grading of oligodendrogliomas. Several grading schemes have been proposed throughout the years in an attempt to standardize diagnosis and develop a system which may also provide prognostic information. Five histomorphologic parameters including high cellularity, nuclear atypia, mitoses, necrosis, and endothelial proliferation have been variably used in different studies to indicate the degree of malignancy of oligodendrogliomas. In 1987,

Burger et. al. reviewed 84 sections of oligodendroglial tumors and evaluated each using 15 different histomorphologic features.[23] In order to attain prognostic information, survival analyses was also performed and five of the 15 histologic features had significant correlation with survival by univariate analysis. In decreasing order of statistical significance, these included: mitoses, necrosis, cytologic atypia, vascular hypertrophy and vascular proliferation.[23] Although results of other studies have highlighted different criteria, Burger's findings emphasize the usefulness of mitoses and necrosis, specifically in differentiating anaplastic subtypes of oligodendrogliomas.[23]

WHO grade II versus WHO grade III oligodendrogliomas

WHO grade II oligodendrogliomas are monomorphous gliomas of moderate cellularity while grade III tumors occasionally exhibit marked cellular pleomorphism with multinucleated giant cells. The majority of anaplastic cells in grade III oligodendrogliomas still have reminiscent oligodendroglial cell properties: rounded hyperchromatic nuclei, perinuclear swelling, and few processes; however, mitoses, microvascular proliferation and necrosis with or without pseudopalisading may also be present.[14]

Mixed oligoastrocytomas

Mixed gliomas are composed of two or more distinct populations of neoplastic macroglial cells. The most common human mixed glioma is the oligoastrocytoma (WHO grade II), but others include the anaplastic oligoastrocytoma (WHO grade III) and the ependymoma-astrocytoma. Oligoastrocytomas are moderately cellular with low mitotic activity. Further subclassification into oligodendroglioma-predominant, astrocytoma-predominant or approximately equal components has been suggested, but no difference in prognosis or response to therapy has yet been identified.[14]

F. Molecular biology and markers of oligodendroglial tumors

Chromosomal changes

Significant advances in the understanding the molecular biology and genetics of oligodendrogliomas has recently been achieved. Oligodendrogliomas demonstrate

distinct genetic changes which differentiate them from other types of gliomas. Specifically, the most common genetic alteration in oligodendroglial tumors is the loss of heterozygosity (LOH) on the long arm, designated "q", of chromosome 19. The incidence of the LOH on 19q is variable, but reported between 50-80% of oligodendroglial tumors. The second most frequent occurrence of genetic alterations is LOH on the short arm, designated "p", of chromosome 1. Reported incidences of LOH on 1p ranges from 40-90%.[24,25] The allelic loss of 1p has also been reported to be a statistically significant predictor of tumor sensitivity to chemotherapy. A combination of 1p and 19q has been statistically associated with a longer recurrence-free survival after chemotherapy. A study by Bauman et.al. found that humans with oligodendroglial tumors and allelic loss of chromosome 1p also predicts longer progression-free survival among patients receiving radiotherapy, with or without chemotherapy. It has since been suggested that cytogenetic studies to assess the integrity of 1p and 19q be added to the diagnostic evaluation of oligodendrogliomas.

Markers of cellular proliferation

In an attempt to provide additional information regarding tumor behavior, cell proliferation markers such as Ki-67 and proliferating cell nuclear antigen (PCNA) have been studied.[26,27] The Ki-67 protein has become the most promising of proliferation indices. Initial investigation revealed the Ki-67 antigen present in the nuclei of cells in the G1, S, and G2 phases of the cell division cycle as well as in mitosis.[28] Quiescent resting cells in the G0 phase do not express the Ki-67 antigen. Since the Ki-67 antigen was found to be present in all proliferating cells including normal and tumor cells, it has since been applied as a marker to determine the growth fraction of a given cell population.[28]

The usefulness of the Ki-67 labeling index has been well established for various types of malignant neoplasms. In cases of multiple myeloma, it has been shown that Ki-67 expression correlates with the course of the disease and is a useful marker in distinguishing multiple myeloma from other monoclonal gammopathies of unknown significance.[28]

Several human studies have evaluated the Ki-67 staining of oligodendrogliomas. Typically, the mitotic activity in WHO grade II oligodendrogliomas is nearly absent. Therefore, the labeling indices for proliferation markers generally are quite low.[29]

Prognostically, the utility of the Ki-67 labeling index has also been demonstrated in human brain tumors. Tortosa et.al. analyzed 95 patients with histologically-confirmed tumors including anaplastic astrocytomas, anaplastic oligoastrocytomas and anaplastic oligodendrogliomas.[30] In this set of anaplastic gliomas, a Ki-67 index of 5.1% or lower was found to be independently associated with longer survival times. Similarly, Reis-Filho et.al. evaluated a population of confirmed WHO grade II oligodendrogliomas for the presence of Ki-67 indices.[27] According to their findings, disease free survival was significantly shorter when tumor expressed Ki-67 index >5%.

Other tumor markers

Another aspect of understanding more about tumor biology involves the investigation of growth factors and their transmembrane receptor kinases. These proteins play important roles in cell proliferation, survival, migration and differentiation. One group of growth factors, comprising epidermal-growth factor (EGF)-like proteins, stimulates cells to divide by activating members of the EGF receptor (EGFR) family.[31] This group of receptors includes the EGFR itself as well as the human epidermal growth factor receptor (HER) classes 2-4. While EGFR induced signaling is essential for many physiologic processes, the aberrant activity of this protein and receptor family also plays an important role in the development and growth of tumor cells. These transmembrane receptors are composed of an extracellular ligand-binding domain and a cytoplasmic region with tyrosine kinase enzymatic activity.[31] This structure allows signals to be transmitted across the plasma membrane where they activate gene expression and ultimately induce cellular responses such as proliferation.

EGFR has become increasingly investigated as a potential diagnostic and therapeutic target in neurooncology. Inhibitors of tyrosine-kinase have become an additional target for reducing tumor burden and prolonging survival in humans.[32] Mellinghoff et.al. reported approximately 20% of patients with glioblastoma multiforme who had increased expression of EGFR and were treated with a tyrosine kinase inhibitor

had at least a 25% reduction in tumor burden.[32] Although more often investigated in cases with astrocytic tumors and even mammary tumors, alterations in EGFR expression have been observed in oligodendroglial tumors. Reifenberger et.al. reviewed a series of WHO grade II oligodendrogliomas and WHO grade III anaplastic oligodendrogliomas for the presence of EGFR mRNA expression by Northern blot analysis as well as EGFR protein by immunocytochemistry.[33] The authors found 6 of 13 grade II tumors and 10 of 18 grade III tumors showed mRNA and protein expression indicating a possible role of tyrosine kinase inhibitors in the treatment of this tumor type as well.

G. Grading and pathology of canine oligodendrogliomas

Classification of oligodendrogliomas in dogs is a vital tool for communication between the veterinary clinician and pathologist. Additionally, establishing a common language of tumor classification allows comparative aspects to be made to assist veterinary and human investigators. The first international histological classification of nervous system tumors in domestic animals was published in 1974.[1] This committee recently revised the classification in 1999 and adopted similar features outlined by the WHO classification scheme.[1] Similar to their human counterparts, canine oligodendrogliomas are generally characterized based on histomorpholgic features and the degree of differentiation in a manner as described for analogous human tumors. This determination aims to ultimately provide a basis for prognosis as well as tumor-specific therapy.

Canine oligodendroglial tumors can be differentiated into oligodendrogliomas, which correlate to WHO grade II oligodendrogliomas in people, and anaplastic oligodendrogliomas, which correlate to human WHO grade III oligodendrogliomas. Canine oligodendroglial tumors may also have neoplastic oligodendrocytes and astrocytes either intermingled or separated into distinct clusters. These “mixed gliomas” or oligoastrocytomas correlate to their human counterpart although further classification into WHO grade II and WHO grade III correlations have not yet occurred in the veterinary classification scheme.[1]

Grossly, oligodendrogliomas appear red, pink-red, or grey-pink and often are soft, gelatinous masses.[1] They predominantly occur in the cerebral hemispheres including

subcortical white matter and often affect the ventricular and meningeal surfaces. Microscopically, WHO grade II canine oligodendrogliomas appear moderately to highly cellular and have uniform nuclear shape and size. Additionally, mitotic figures may be rarely observed. Similar to their human counterparts, canine oligodendroglial tumor cells have hyperchromatic nuclei and lightly-stained cytoplasm forming a perinuclear halo. Distinct cell membrane borders and a "honeycomb" or "fried-egg" appearance can also be observed secondary to autolysis. Mucinous cystic degeneration is common and necrosis is rare. Furthermore, a vascular "chicken-wire" pattern and cellular arrangements in rows within the white matter is sometimes observed.[1]

Canine anaplastic oligodendrogliomas, which correlate to WHO grade III, are defined by higher cellularity, prominent proliferation of glomeruloid vessels, nuclear polymorphism, increased mitotic index, necrosis, and/or meningeal infiltration.[1] Other occasionally observed findings include intermingled astrocytes and multinucleated giant cells.[35]

Canine mixed oligoastrocytomas, although yet to be further definitively classified, are diagnosed if the glial tumor exhibits neoplastic oligodendrocytes and astrocytes either intermingled or geographically distinct.[1] At times, anaplastic oligoastrocytomas exhibit such profound vascular proliferation and necrosis, differentiation from a high-grade, anaplastic astrocytoma or glioblastoma multiforme can be extremely difficult.[1]

H. Prevalence of canine oligodendrogliomas

Overall, glial tumors are considered relatively common primary tumors of the CNS in dogs, second only to meningiomas.[34] Of these neoplasms, astrocytomas comprise the majority of canine gliomas in the United States, whereas oligodendrogliomas are more frequently diagnosed in Switzerland and other European countries.[34] In a recent retrospective review of canine intracranial tumors, of 173 primary brain tumors evaluated at a US veterinary referral hospital, 45% were meningiomas compared to 17% astrocytomas and only 14% oligodendrogliomas.[35] Canine intracranial oligodendrogliomas occur most commonly in brachycephalic breeds, particularly Boxers and Boston Terriers. Fifty-percent of all gliomas occur in these breeds; however a genetic basis for this occurrence has not yet been identified.[1] A gender predisposition is

not apparent in canine oligodendrogliomas. Additionally, oligodendrogliomas are recognized with greater incidence in dogs greater than 6 years of age and are rare in immature dogs (i.e., less than 6 months of age).[34] Keller et al. reviewed neoplasms of 69 immature dogs. Although intracranial neoplasia was determined to be the second most common location of tumors affecting these immature dogs, only three of 69 tumors were gliomas.[36]

One of the earliest case reports of a dog with an oligodendroglioma was reported in 1972 which affected a 14 year old Boston Terrier.[37] Since that time, single case reports describing intracranial oligodendroglial tumors include a 15-month old Golden Retriever, 5 year old French Bulldog, and a 12 year old Boxer with a simultaneously occurring meningioma.[38-40]

I. Clinical features of canine oligodendrogliomas

Clinical signs of canine oligodendrogliomas vary depending upon the location of the tumor within the neuraxis. These neoplasms are space-occupying lesions which can affect the surrounding parenchyma by multiple mechanisms including compression, infiltration, and parenchymal destruction. Infiltration and parenchymal destruction may occur leading to secondary effects of peritumoral edema, hemorrhage, necrosis and reactive inflammation. Hydrocephalus may also be a secondary effect if the flow of cerebrospinal fluid is obstructed by the tumor.[34] Oligodendrogliomas can also arise from the spinal cord parenchyma. Both reported cases of spinal oligodendrogliomas in dogs identified the tumor arising intramedullary within the cervical spinal cord and exhibited neurologic deficits of a typical cervical myelopathy.[41,42] The single case report of an oligodendroglioma affecting a bull was also identified in the cervical spinal cord at the time of post-mortem examination.[43] It is appears that extraaxial metastasis of oligodendrogliomas is rare. In a pathology study of 18 dogs diagnosed with oligodendrogliomas that had thoracic radiographs performed, none had evidence of overt metastasis.[35]

Foster et al. reviewed the clinical features of 43 dogs with tumors affecting the rostral cerebrum.[44] Seven (16%) of these tumors were gliomas based on necropsy findings although all were diagnosed as astrocytomas. These dogs were all determined to

have either neurologic deficits (abnormalities of proprioception, sensation, vision, or motor initiation) or neurologic signs such as seizures or behavioral changes (dementia, aggression, alterations of established habits). In another study of canine brain tumors, all dogs with oligodendrogliomas initially exhibited seizures and/or behavior changes while none were found to have neurologic deficits alone. Five of these dogs later developed interictal neurologic deficits within a mean of 82 days.[44]

The clinical signs of 97 dogs with brain tumors were reported by Bagley et al.[45] Two of the tumors evaluated by biopsy or necropsy evaluation were oligodendrogliomas.[45] Although the clinical sign associated with specific tumor type was not determined, most dogs (76%) had tumors in the supratentorial region (cerebral hemispheres, basal nuclei and diencephalon). Seizures were the most common sign at initial examination, affecting 45% of all dogs with brain tumors in this study. Other signs included circling, ataxia, nonspecific behavior changes and aggression.[45]

Cervical spinal hyperesthesia has also been recognized as a clinical sign of intracranial disease.[46] One of 8 cases reported with this finding was a Boston terrier with a glioma of the left cerebrum. Secondary hydrocephalus and foramen magnum herniation were identified during post-mortem examination. The etiology of this clinical sign is unknown, but postulated to be secondary to displacement of cranial or cervical nerves directly by the mass or indirectly by increased intracranial pressure secondary to obstruction of CSF flow. In another case report, an oligodendroglioma was diagnosed at necropsy in a dog exhibiting hyperresponsiveness and hyperesthesia as the dominant neurological findings.[47] The authors proposed similar pathomechanisms as the previous case series.

J. Clinicopathologic and diagnostic evaluation of canine oligodendrogliomas

Although there are common clinical signs in dogs with oligodendrogliomas, these findings are generally not tumor specific. Various diagnostic modalities have been applied in dogs with intracranial neoplasia in order to correlate specific characteristics with the tumors. Steiss et al. examined 8 dogs with various intracranial tumors and performed electroencephalograms (EEG) on each.[48] On necropsy examination, no dog was diagnosed with an oligodendroglioma, but four dogs were identified with gliomas (1

astrocytoma, 1 undifferentiated glioma, 2 mixed gliomas). No EEG pattern was pathognomonic for tumor type or tumor location. High-voltage slow wave activity was seen in 2 and low-voltage fast wave activity was observed in 2. All dogs were noted to have cerebral edema, elevated intracranial pressure, secondary hydrocephalus and displacement of brain structures possibly affecting the EEG recordings.[50] Overall, EEG is a nonspecific modality for brain tumor diagnosis.

The use of brain scintigraphy has also been applied to dogs with intracranial tumors, although this modality is rarely used in modern clinical practice due to its inherent invasiveness and low specificity. Kallfelz et al. examined 21 cases of suspected intracranial disease.[49] No dog was found to have an oligodendroglioma; however two of these dogs had an astrocytoma in the rostral cerebrum at necropsy. During scintigraphic evaluation, both cases revealed increased uptake of the radiopharmaceutical in the region affected, suggesting abnormal soft tissue within the cranial vault.[51] However, cerebral neoplasia could not be distinguished from an inflammatory lesion with this diagnostic tool. Further comparative analysis to distinguish scintigraphic features between disease types was not performed.

Bailey et al reviewed the results of complete cisternal cerebrospinal (CSF) analyses of 53 dogs with primary brain tumors.[50] Three dogs were diagnosed on post-mortem examination with oligodendrogliomas. One dog had normal CSF findings. The other 2 had white blood cell counts <50 cells/uL with elevated protein content (>25 mg/dL) and normal CSF pressure (<170 mm CSF) supporting the poor sensitivity of CSF analysis with respect to diagnosis of oligodendrogliomas and brain tumors in general. Further correlations between certain CSF characteristics and histologic features or location of the tumors could not be elucidated. The authors then proposed that other factors such as the biologic behavior of the tumor or stage of an animal's illness likely also influence CSF abnormalities.[50]

Other diagnostic tools have been explored for the antemortem identification of canine gliomas. Gallagher et al. reviewed the use of ultrasonography in 15 dogs and cats with brain or spinal disease.[51] This modality was strictly utilized intraoperatively and performed in 1 dog with a glioblastoma who underwent a craniotomy. The mass appeared ill-defined and homogenously hyperechoic; and histologically, contained

smaller amounts of mineralization. Although the lesions were readily imaged, specific ultrasonographic criteria for the tumor could not be determined when compared with the other tumor types examined.[53]

Fine-needle aspiration of canine brain tumors has also been evaluated as a method for obtaining a cytologic diagnosis. Of 10 dogs evaluated by Platt et al., 2 had oligodendrogliomas confirmed histologically.[52] This diagnosis was achieved in both cases with a needle biopsy. Although cytologic diagnosis was accurate in both cases compared to the needle biopsy, other tumor diagnoses varied considerably leading the authors to advocate additional methods such as CT-guided biopsy for a more accurate representative sample.[54]

A variety of stereotactic CT-guided brain biopsy (SCTGBB) devices have been evaluated for the diagnosis of brain lesions. Moissonnier et al. initially determined the accuracy of a modified Laitinen's stereoadapter in 10 normal canine cadaver heads.[53] The authors concluded that the device could accurately sample a mass at least 6mm in diameter. This device was then applied to 23 client-owned dogs with intracranial masses on CT examination.[54] The study compared cytological and histopathological diagnoses as well as evaluated early postoperative complications associated with the procedure. Eight of 23 dogs were diagnosed with gliomas using both conventional methods including five diagnosed with oligodendrogliomas. There was also agreement between cytologic and histopathological diagnoses in all cases. Complications were observed in 6 patients including wound hemorrhage and seizures. Three of these dogs were diagnosed with gliomas. Perioperative coma and death occurred in 2 dogs with brainstem gliomas.[56]

Koblik et al also evaluated a SCTGBB device using the modified Pelorus Mark III Stereotactic System.[55] The authors initially modified the device for application of the canine skull and found the mean needle placement error was significantly related to location of the target lesion. Lesions located within the caudal fossa caused the largest mean error of needle placement compared to those in the middle and rostral fossa.[55] The authors also evaluated the device in 50 client-owned dogs with intracranial lesions.[56] Gliomas were categorized as "other neoplastic lesions" and the accuracy of diagnosis using the SCTBB device compared to standard surgical/necropsy methods was

performed. Four of 22 dogs were grouped in this category and only 2 of these yielded a correct diagnosis. A total of 5 dogs experienced post-operative complications. One dog with a glioma had seizures immediately post-biopsy, became obtunded, and died 1 day later.[58]

A disposable real-time SCTBB device was also evaluated by Flegel et al.[57] The system was studied using 2 cadaver dogs and 2 anesthetized dogs with normal brains. Although the device was reportedly accurate and fast for biopsy acquisition, lateralizing forebrain deficits were present in 1 dog following the procedure.[59]

Giroux et al introduced the design, construction and accuracy of another SCTBB device for the canine brain.[58] Although not applied to client-owned animals, this device was assessed using needle placement into the pituitary gland and caudate nucleus. Accuracy of the device in targeting these two regions were 98.6% and 75%, respectively. The authors also recognized the benefit of this device with regards to cost of construction compared to modified human prototypes.[60]

K. Diagnostic imaging features of canine intracranial neoplasia

Imaging of intracranial neoplasms has been an evolving and well studied aspect of neurooncology for the past 20 years. Bailey reviewed the historically performed diagnostic imaging procedures of intracranial lesions including survey radiography, ultrasonography, cerebral angiography (cavernous sinus venography, subtraction radiography, internal carotid angiography, vertebral-basilar angiography) and thecography.[61] These techniques have been replaced by CT and MR imaging due to several limitations including restricted visualization of cranial vault lesions, inherent invasiveness, risk of complications, and species restrictions.[59]

The diagnosis of canine gliomas was first recognized using CT imaging by Swengel in 1982.[62] This case report of a Boxer with a suspected glioma was based on the CT findings including an intra-axial right cerebral mass with ring-enhancement following intravenous contrast administration. Severe hydrocephalus of the left lateral ventricle was also observed. The dog was diagnosed with a mixed oligoastrocytoma following necropsy examination.[60]

Turrel et al reviewed the CT characteristics of a variety of primary brain tumors in 50 dogs.[61] Thirteen dogs were diagnosed with gliomas following surgical biopsy or necropsy examination. Distinguishing between astrocytomas and oligodendrogliomas by CT was difficult since both tumor types had similar intra-axial appearances including nonuniform ring enhancement, as well as poorly-defined tumor margins. Although the authors did not evaluate the accuracy of CT diagnosis, features such as location, uniformity or degree of enhancement helped differentiate gliomas from other tumor types.[63]

MRI is now the preferred modality for imaging humans with neurologic disease and has become increasingly available and affordable in veterinary medicine. Compared to CT imaging, MRI provides improved contrast resolution, detailed depiction of anatomic structures in all regions within the cranial vault and allows differentiation of edema and/or hemorrhage from neoplastic tissue. Kraft et al reviewed the MRI appearance of 50 primary brain tumors in dogs.[62] A total of 9 gliomas were assessed and compared with other tumor types. Two oligodendrogliomas appeared as intra-axial T2-hyperintense, T1-hypointense or isointense, varied in degree of peritumoral edema (none to severe) and varied in degree of contrast enhancement (none to strong with ring-enhancement).[64] Although some features were common among different tumor types including presence of hemorrhage and mineralization, the tumor location (i.e., intra-axial versus extra-axial) appears most useful for distinguishing glial tumors from other tumor types.

The correlation of CT and MRI findings with histopathologic findings has also been evaluated.[63] Two of 10 dogs studied were diagnosed with gliomas via histopathology following intracranial imaging. One dog underwent CT imaging and was presumed to have an extra-axial, well-demarcated mass with marked homogenous enhancement. The imaging differential diagnosis was a meningioma although a gemistocytic astrocytoma was found at necropsy. The other dog had MRI imaging performed and found to have an intra-axial mass of the temporal lobe with marked enhancement and invasion of adjacent structures. The imaging and histological diagnosis agreed the tumor was a glioma in this dog. Overall, MRI correlated with histopathology

in all cases while CT imaging correctly identified the histological tumor type in six of seven dogs.[65]

Differentiating glial tumors from non-neoplastic tissue has also proved challenging. The utility of MRI for distinguishing between these two lesions has been studied.[64] Six of 30 dogs with primary brain neoplasms were diagnosed with gliomas and their MRI features were compared with those from 16 dogs with non-neoplastic diseases. Signs including solitary lesion, regular shape, mass effect, dural contact and dural tail, and contrast enhancement were significantly more likely to be seen with neoplastic diseases.[66] Meningiomas were over-represented in the neoplastic lesion category and may have influenced the observed associations.[66]

L. Treatment and survival of canine oligodendrogliomas

Little data concerning the influence of various palliative or definitive treatments on the survival of dogs with oligodendrogliomas currently exists. Foster et al. reported on the clinical course of disease in 43 dogs with rostral cerebral neoplasia.[44] Seven of 43 dogs were diagnosed with gliomas and the time from initial neurologic signs to necropsy evaluation ranged from 7 to 150 days with a mean of 77 days. No information regarding type or duration of treatment administered to these dogs was provided.[46]

In another study, a median survival time of 56 days was reported for dogs with brain tumors treated only symptomatically with corticosteroids and anticonvulsant therapy.[61] Of these 8 dogs with various tumor types, 1 dog was diagnosed with an oligodendroglioma and survived 64 days. The remaining dogs in this study were diagnosed with other tumor types and treated with fractionated megavoltage radiation. The overall median survival time in this study of irradiated dogs versus symptomatically treated dogs was significantly longer (322 days versus 56 days, $p<0.05$).[67] Radiation was also the only treatment for 10 dogs diagnosed with gliomas in another report.[61] The median survival time of this group was 176 days. There was no distinction between animals with oligodendrogliomas or other gliomas in this paper.[63]

Heidner et al. examined the effect of various treatment modalities on survival in dogs with brain tumors.[65] Gliomas were seen in 7 of 86 dogs reported and included in a non-meningioma group for statistical evaluation. Other tumor types classified as non-

meningiomas included choroid plexus tumors (6 dogs) and secondary brain tumors (17 dogs). The median survival time of non-meningiomas was significantly shorter than the meningioma group (0.3 versus 1.6 months, p= 0.001).[68] Treatment type for all tumors was also statistically evaluated and cobalt-60 radiation therapy with or without other treatments was found to provide the best survival times, compared with surgically-treated (with or without radioactive iodine 125 implants) and with symptomatically treated groups (4.9 months versus 0.9 and 0.2 months, respectively, p< 0.001).[68]

The role of chemotherapy for treatment of canine oligodendrogliomas has received little attention. A case report describes a Boston Terrier with a suspected astrocytoma based on CT imaging.[66] The dog was treated with phenobarbital (1mg/kg PO BID), prednisone (0.5mg/kg PO SID) and carmustine (50 mg/m^2 body surface area every 6 weeks). The authors also reported an objective decrease in tumor size based on repeat imaging attributed to the chemotherapeutic agent. The dog's clinical status obtained partial remission for a period of 7 months and the tumor type was confirmed histologically following necropsy examination.[69]

M. Immunocytochemistry and canine brain tumors

To date, little data currently exists regarding tumor biology of spontaneous canine brain tumors. Several methods have been implemented in human neurooncology which may translate to the veterinary field. These include the investigation for underlying alterations of mRNA using such methods as polymerase chain reaction (PCR) and reverse-transcriptase PCR (RT-PCR) as well as DNA changes using comparative genomic hybridization (CGH) and fluorescence *in situ* hybridization (FISH).[67,68] In humans, immunocytochemistry has also been employed to investigate altered protein expression adding vital information regarding tumor progression. Immunohistochemical stains were performed using neuroblastoma tissue in a 13 year old dog following necropsy evaluation.[69] In this report, the authors utilized the neuronal markers, neuron-specific enolase (NSE), synaptophysin and neurofilament protein (NFP), in order to identify the tumor possessing neuronal differentiation. Another epithelial marker, cytokeratin, was also used in order to distinguish the primitive neuroectodermal tumor

from a neuroendocrine tumor.[72] In this manner, immunohistochemical markers are useful for diagnostic purposes.

Another application of immunohistochemistry includes its use in differentiating tumor grades within specific histologic types of tumors. For example, choroid plexus carcinomas from four dogs were examined using several immunohistochemical markers.[70] Examination of labeling patterns showed differences for immunoreactivity of the tumor for several epithelial markers including pancytokeratin, cytokeratin AE1 and vimentin, between well-differentiated and poorly-differentiated choroid plexus carcinoma.[73] The authors subsequently proposed possible phenotypic subtypes of this tumor in the dog.

Similarly, Barnhart et.al. investigated the immunocytochemical staining patterns of several meningioma variants from 15 dogs.[71] The immunoreactivity were correlated with the tumor subtype, and the authors noted the two most common meningeal variants, meningothelial and transitional, appeared to have differences in cytokeratin expression. They also suggested canine meningiomas have antigenic expression comparable to their human counterparts.[74]

Canine intracranial meningiomas have also been used to investigate angiogenesis and vascular permeability as related to tumor growth. Platt et. al. found that expression of vascular endothelial growth factor (VEGF) was detected in 17 of 19 surgically-resected meningiomas, with more than 50% of cells staining positively.[52] Although there was trend toward shorter survival times with greater VEGF expression, the intensity and distribution patterns of the stain were not significantly associated with survival.[75]

Immunocytochemistry using canine gliomas have received less attention, as only four publications regarding this topic currently exist. Lipsitz et.al. reported immunocytochemical findings in five post-mortem cases of canine glioblastoma multiforme.[72] The authors reported the strong GFAP immunoreactivity in all cases; however other immunohistochemical markers including VEGF and EGFR showed variable reactivity (40% and 60% of the five cases respectively),. In this study, patterns of EGFR and VEGF immunoreactivity were similar to those found in the human glioblastomas, suggesting that similar processes of tumor growth such as deregulation of genes involved in cell growth control and angiogenesis occur in both species.[76]

Similarly, Stoica et.al. aimed to further characterize astrocytomas in dogs and to determine whether there are genetic changes comparable to those identified in human medicine.[46] Specifically, the recognition in human astrocytomas of the loss of wild-type p53 activity, a gene normally responsible for tumor suppression, relates to increased genomic instability. Secondarily, accelerated neoplastic progression is noted leading to tumor growth and undifferentiation.[46] The authors investigated the p53 gene sequence from 12 of 31 dogs with astrocytomas using PCR, but identified a DNA mutation in only one case. All 31 tumors were stained for p53 using immunocytochemistry with nuclear staining greater than 10% of counted cells being considered as exhibiting p53 overexpression.[77] Notably, 35% of the astrocytomas had evidence of p53 protein overexpression, leading the authors to suggest other mechanisms besides upregulation of p53 or presence of a mutation to explain the disagreement with their findings. Such explanations include genotoxic stress or additional genetic alterations; i.e., mutations affecting other exons than those investigated.[77]

The use of immunocytochemistry in canine oligodendrogliomas has only been reported in one instance. Vandevelde et.al. reported immunocytochemical staining findings of 74 canine brain tumors including 11 oligodendrogliomas.[73] Three stains were applied to this tumor type including GFAP, myelin-associated glycoprotein (MAG) and myelin-basic protein (MBP). The results indicated absence of GFAP positive tumor cells in all canine oligodendrogliomas which at the time, was consistent with similar findings in man.[78] Additionally, the authors investigated the expression of MAP since it is known to be a marker of undifferentiated oligodendrocytes in brain tissue culture.[74] They found only 3 of 11 oligodendrogliomas expressed MAG and considered such technical features as paraffin-embedding, which can cause artifactual alteration of cytoplasm within tumor cells (and also accounts for the typical honeycomb appearance), accounted for the low percentage of MAG expression. Also, no oligodendroglioma expressed MBP consistent with a previous report which found no MBP expression in oligodendrocytes grown in tissue culture.[78]

Canine oligodendrogliomas were also included in a recent review of primary brain tumors when investigating for growth factors involved in angiogenesis.[75] Higgins et. al. performed tissue microarray immunophenotyping to evaluate expression of EGFR

and platelet-derived growth factor receptor alpha (PDGFRα) in 3 WHO grade II canine oligodendrogliomas and 37 WHO grade III oligodendrogliomas.[75] Overexpression of PDGFRα was observed 86% of the grade III tumors while no overexpression was seen in grade II tumors. EGFR overexpression in this series was seen in only 3% of grade III oligodendrogliomas and no grade II oligodendrogliomas. The authors suggested close parallels of overexpression in the human counterparts. Dickinson et.al. also reported the use of real-time PCR to evaluate the expression of mRNA for five tyrosine kinase growth factor receptors including VEGFR-1 and 2, endothelial growth factor receptor-1 (EGFR-1), PDGFRα and c-Met. Product was amplified from all 20 oligodendrogliomas histologically classified as both WHO grade II and WHO grade III.[80] Increased mRNA expression of PDGFRα was mostly restricted to this tumor type compared with the other tumors investigated including all WHO grades of meningiomas and astrocytomas. Comparisons between oligodendroglial tumor grades were not performed in this study. The authors cited one reference demonstrating increased expression of PDGFRα in human oligodendrogliomas and subsequently theorize comparable biological behavioral between human and canine tumors. As further investigations of such growth factors involved with tumor growth or behavior continue, similar findings will help to validate the role of growth-factor targeted therapies in the treatment of these neoplasms.

Chapter 2 – Materials and Methods

Tissue Source

Fifteen dogs with spontaneous oligodendroglial tumors were studied. Formalin-fixed, paraffin embedded tumor blocks and hematoxylin and eosin (H&E) stained sections on glass slides of tumors were retrieved from the archives of two veterinary pathology laboratories, including the Virginia-Maryland Regional College of Veterinary Medicine at Virginia Tech (3 dogs) and University of Tennessee College of Veterinary Medicine (12 dogs). All samples were derived from clinical cases submitted for necropsy examination.

Clinicopathologic Material

The case records of the 15 cases diagnosed with oligodendroglial tumors were reviewed and the following information was retrieved: breed, age at the time of necropsy, sex, initial histomorphologic diagnosis, and tumor location.

Histomorphologic Evaluation

Hematoxylin and eosin (H&E) stained sections of each tumor were reviewed by the primary investigator (MAH), one board-certified veterinary anatomic pathologist (BSJ) and one human neuropathologist (CS). Both pathologists were blinded to any clinical information, diagnosis made at the time of necropsy, and diagnosis made by the other pathologist. Oligodendroglial tumors were diagnosed and graded according to standard WHO criteria (i.e., WHO grade II or WHO grade III).[8] In cases where pathologists visualized two distinct glial cell populations (oligodendroglial and astrocytic), a diagnosis of a mixed glioma (i.e, oligoastrocytoma) was made. Oligoastrocytomas were then graded (WHO grade II or WHO grade III) using WHO criteria.[8] When discordance between observers regarding the histomorphologic diagnosis was present, slides were re-reviewed, with the final diagnosis representing a consensus among at least 2 of the three observers.

Sections stained with H&E were then specifically evaluated for five histomorphologic variables, including necrosis, mitoses, vascular hypertrophy, cellular atypia, and vascular proliferation. Necrosis and vascular proliferation were evaluated as

qualitative variables which were either present or absent. Vascular hypertrophy was defined as endothelial cells that were enlarged but did not appear to be increased in number. Vascular proliferation was defined as endothelial cells which were increased in number. Vascular proliferation was graded as absent, present or present and marked. Cytologic atypia was evaluated and graded using a semi-qualitative scale: none, slight, moderate, or marked. The mitotic rate was determined by the counting the number of cells in metaphase in five high power fields (hpf; 100X objective). Grading of mitoses was then reported as none/5 hpf, 1-5/5 hpf, 6-10/5 hpf, 11-20/5 hpf, and 21-30/ 5 hpf.

Immunocytochemical Techniques and Analyses

The indirect peroxidase-anti-peroxidase technique using an automated avidin-biotin slide staining system (Ventana Benchmark LT, Ventana Tucson, AZ) was used as previously described.[46] Tissues were deparaffinized and incubated in a moist chamber at room temperature with a series of xylene and gradient alcohols to water and appropriate buffers. Deparaffinized tissue sections (5 μm thick) from each tumor were incubated with primary antibodies against GFAP (GFAP-rabbit polyclonal 760-2516, 1:50 dilution, 16 minutes incubation; Ventana, Tucson, AZ), EGFR (EGFR-rabbit polyclonal PU335-UP, 1:100 dilution, 32 minutes incubation; BioGenex, San Ramon, CA), and Ki-67 (Ki-67-mouse monoclonal 790-2910, 1:50 dilution, 16 minutes incubation; Ventana, Tucson, AZ). Goat amti-rabbit and anti-mouse IgG-biotin (1:100 dilution, 16 minutes, Ventana, Tuscon, AZ) served as secondary antibodies. Before mounting, the sections were counterstained with hematoxylin for 2 minutes. For negative control slides, a negative reagent control or rabbit negative control was substituted for the primary antibody. Positive controls for GFAP included immunostaining of the glia limitans, as well as a tissue section from a human glioblastoma multiforme used in parallel. A positive control for EGFR was staining of canine epidermis. A normal canine lymph node was used the positive control for Ki-67 immunostaining.

Quantitative morphometric analyses

A quantitative image analysis system (Adobe Photoshop version 7.0, Adobe Systems, Inc., San Jose, CA) was used to analyze morphometric data and calculate

percentage of Ki-67 immunoreactivity. Images of selected tumor regions were chosen from areas of increased staining uptake (one selected region per tumor), and were digitally captured using the 40X objective of a light microscope and a digital camera. Images were displayed on a personal computer and a customized software macro was used to count the total number of tumor cells demonstrating immunoreativity to Ki-67, as well as the total number of tumor cells in the image. A minimum of 1000 total tumor cells were evaluated, and Ki-67 immunoreactivity subsequently expressed as the percentage of tumor cells with positive Ki-67 compared to the total number of tumor cells present.

Qualitative morphometric analyses

GFAP immunoreactivity was assessed in each tumor as either qualitatively absent or present. EGFR immunoreactivity was then assessed in each tumor as either absent, mild, moderate, or marked. The intensity of staining for each tumor was subjectively scored by comparing tumors between groups of staining patterns. For qualitative immuncytochemical analyses, all 15 tumors were assessed at least three times.

Statistical analyses

For categorical data, multi-way contingency tables were constructed to assess associations between severity of tumor and patterns of their morphological characteristics. Due to a large number of cells with counts (frequencies) ranging from 0 to 4, only exact statistical tests computed by a statistical software program (StatXact 7, Cytel Software, Cambridge MA) were used in the analysis. Associations between grades of tumor and dichotomous morphological characteristics such as necrosis, vascular hypertrophy, and GFAP immunoreactivity were assessed using the normal scores test, while associations between grades of tumor and ordinal morphological characteristics such as mitoses, cellular atypia, vascular proliferation, and EGFR immunoreactivity were assessed using the linear-by-linear association test. Analyses for only continuous outcome (percentage uptake of the Ki-67 stain) were performed using a statistical software program (SAS, version 9.1, SAS, Cary, NC). Medians as well as interquartile ranges were computed and compared between groups using the exact Kruskal-Wallis test

followed by two comparisons using the exact Wilcoxon rank sum test. Statistical significance was set at alpha= 0.05 for each of the initial tests and at alpha= 0.0167 for any subsequent multiple comparison tests.

Chapter 3 – Results

Patient and Tissue Source Data

Fifteen tumors were included in the study. Examining pathologists ultimately diagnosed 6/15 tumors as WHO grade II oligodendrogliomas, 5/15 as WHO grade III oligodendrogliomas, and 4/15 as WHO grade II oligoastrocytomas (Table 1; Appendix 1-Figures 1.1-1.3).

The signalment, tumor location, morphologic tumor diagnosis, and immunocytochemical features of each tumor are summarized in Table 1. The mean age of dogs at the time of diagnosis was 8.1 $\pm$ 0.84 years (range, 3 to 12 years). Breeds (Table 1) included the Boston Terrier (5), Boxer (4), English Bulldog (1), Bullmastiff (1), American Staffordshire Terrier (1), Labrador Retriever (1), Miniature Schnauzer (1) and mixed breed (1). There were 6 neutered males, 3 intact males, 5 spayed females and 1 intact female included in the study (Table 1). The tumors were located within the cerebral hemispheres in 13/15 dogs (5 right-sided, 8 left-sided) and brainstem in 2/15 (Table 1).

Histomorphologic Tumor Characteristics

Results of histomorphologic evaluations and overall associations of histomophologic variables with tumor type are presented in Table 2. Overall associations between patterns of vascular hypertrophy, vascular proliferation, and necrosis and tumor type were not statistically significant ($p > 0.05$; Table 3; Figures 3a, 3b, 4a, and 4b). Significant overall associations were found between tumor type and mitoses ($p = 0.012$; Table 2) and cellular atypia ($p = 0.02$; Table 2).

Multiple comparisons showed that the number of mitoses were significantly greater ($p = 0.002$; Figures 5a and 5b) and cellular atypia more marked ($p= 0.002$; Figures 6a and 6b) in Grade III than Grade II oligodendrogliomas (Table 3). Mitoses and the degree of cellular atypia were not significantly different between grade III tumors and oligoastrocytomas ($p > 0.0167$; Table 3).

Immunocytochemistry

A summary of the immunoreactivity to GFAP, EGFR, and Ki-67 for each individual tumor is provided in Table 1. Immunocytochemical findings included 9/15 tumors which demonstrated immunoreactivity to GFAP (Figures 7a and 7b), and 6/15 tumors were GFAP negative (Table 1). EGFR immunoreactivity was absent in 2/15 tumors, mild in 5/15, moderate in 3/15, and marked in 5/15 tumors (Table 1; Figures 8a and 8b). No significant associations were found between overall GFAP or EGFR immunoreactivity and tumor type ($p > 0.05$; Table 1; Appendix 1) The median percentage of Ki-67 immunoreactivity was significantly different between all tumor types and grades ($p < 0.05$; Figures 1, 9a, 9b, and 9c), and was significantly higher (9.34%) in Grade III oligodendrogliomas than in oligoastrocytomas (6.74%; $p = 0.014$) or Grade II oligodendrogliomas (3.18%; $p = 0.006$; Figure 1). The median percentage of Ki-67 immunoreactivity was also significantly higher in oligoastrocytomas (6.74%) than in Grade II oligodendrogliomas (3.18% $p = 0.014$; Figure 1).

Chapter 4 – Discussion

In this study, 15 spontaneous canine tumors of oligodendroglial origin were evaluated to determine if histomorphologic criteria including necrosis, mitoses, vascular proliferation, vascular hypertrophy, and cellular atypia or GFAP, EGFR, or Ki-67 immunoreactivity were associated with tumor type or grade. The signalment and neuroanatomic distribution of tumors seen in the population of dogs studied are similar to previous reports of dogs with naturally occurring glial neoplasms.[35] In the dogs reported here, 12/15 dogs were brachycephalic breeds including Boxers and Boston Terriers, and previous authors have recognized a predisposition for oligodendroglial tumors in these breeds. Also, our results support those of other investigators in that dogs with gliomas are often middle aged at the time of diagnosis.[34,38-40]

The cellular origin of oligodendrogliomas remains a subject of debate. One possible theory suggests that an early multipotent progenitor cell undergoes mutation, neoplastic transformation and subsequent proliferation.[6] The fact that this progenitor line may normally divide into both astrocytes and oligodendrocytes may explain one possible mechanism for oligodendroglial tumors which display features of astrocytic differentiation, as well as the underlying processes behind the growth of mixed gliomas. An alternative theory of oligodendroglial origin involves the transformation of an immature oligodendroblast lineage. These progenitor cells, although unipotential, have been demonstrated when neoplastically transformed to form both oligodendroglial and astrocytic lineages. A third pathway for the development of oligodendrogliomas may involve the transformation of mature oligodendroglial lineages. Experimental models have demonstrated epitopes of neoplastic cells which are unique to mature, differentiated oligodendrocytes. Other mechanisms for neoplastic glial differentiation and de novo progression of oligodendrogliomas remain to be identified. Since oligoastrocytomas are well-recognized in humans and these tumors contain a clear population of neoplastic oligodendrocytes, we elected to include the mixed gliomas cases in this study in order to compare their morphologic criteria and immunostaining patterns with pure oligodendrogliomas.

Several histomorphologic variables have been shown to be significant prognostic indicators in humans with oliogodendrogliomas.[23] These include, in decreasing order of

importance, mitoses, necrosis, cytologic atypia, vascular hypertrophy and vascular proliferation. We thus elected to apply the same criteria to canine oligodendroglial tumors in order to compare these factors with varying grades of malignancy. In contrast to reports in people, only mitoses and cellular atypia were significantly different between tumor grades in this study. Our results demonstrate that Grade III oligodendrogliomas and oligoastrocytomas both have the potential to contain high numbers of mitoses, while Grade II oligodendrogliomas are characterized by low numbers of mitoses. Similarly, we found that slight or no cellular atypia was a significant feature of Grade II oligodendrogliomas, while Grade III oligodendrogliomas and oligoastrocytomas both contained moderate to marked cellular atypia that was not significantly different between the two tumor types. Thus, this study demonstrates that low numbers of mitoses and the absence of cellular atypia are useful features in the identification of Grade II oligodendrogliomas. These findings should not be surprising, as higher mitotic rates and loss of cellular differentiation are the pathologic hallmarks of high-grade, biologically malignant neoplasms. Of the histomorphologic criteria examined, there were none that aided in the differentiation of high grade oligodendrogliomas and oligoastrocytomas. However, the results of this study offer the veterinary pathologist more insight into oligodendroglial tumor diagnoses. Based on our results, differentiation of WHO grades II and III oligodendrogliomas should be made using mitotic figures and degree of cellular atypia. Since no criteria were statistically significant for differentiating pure oligodendrogliomas from oligoastrocytomas, we also emphasize the importance of the identification of a glial cell subpopulation. The criteria used in our study for an oligoastrocytoma diagnosis included either a geographically distinct or intermingled populations of both glial cell types. These same criteria should also be employed by the veterinary pathologist.

These observed differences in the association of histomorphologic variables and tumor grade between human and canine oligodendroglial tumors may be due to several factors. Our limited sample size in this study is likely due to this uncommon tumor type, but nevertheless should be considered when correlating human and canine variables. Regional heterogeneity is also a well noted feature of high-grade gliomas, and could have

resulted in some bias during sample preparation if certain tumors were, for example, cut into a necrotic core while others were not.

The previously discussed theories on the origin of oligodendroglial tumors help explain why the diagnosis of such tumors can be complex when using standard histopathologic stains and techniques. Although well differentiated oligodendroglial tumors often display classic histomorphologic characteristics such as a "honeycomb" appearance with uniformly round nuclei and swollen, clear cytoplasm, branching capillaries in a "chicken-wire" fashion, and cystic or mucoid degeneration, these characteristics are not pathognomonic for oligodendrogliomas. In humans, oligodendrogliomas may be misdiagnosed as other tumors, including glial and neuronal tumors such as clear cell ependymomas, central neurocytomas and dysembryoplastic neuroepithelial tumors. All of these tumors have the potential to exhibit a similar round nucleus and clear cytoplasm, and can mimic oligodendroglial neoplasms.[8]

The application of Ki-67 immunostaining to canine oligodendrogliomas was performed for several reasons. Briefly, the Ki-67 antigen is present in the nuclei of cells in the G1, S, and G2 phases of the cell division cycle as well as in mitosis.[28] Quiescent resting cells in the G0 phase do not express the Ki-67 antigen. Since the Ki-67 antigen was found to be present in all proliferating cells including normal and tumor cells, it has since been applied to become a marker to determine the growth fraction of a given cell population and has been well-correlated with a tumor's mitotic index. When stained with H&E, oligodendrogliomas may exhibit uniformly darkly stained nuclei, which may mask the detection of mitotic figures. Also, comparing the median labeling index between grades of tumors now offers further clues to the growth and biologic tumor behavior of this tumor type. Specifically, we identified significant difference in the median Ki-67 labeling index between all tumor types examined. WHO grade II oligodendrogliomas had a significantly lower median labeling index compared to grade II oligoastrocytomas. WHO grade III oligodendrogliomas demonstrated the highest median Ki-67 labeling index. Based on the results reported here, routine application of Ki-67 immunocytochemistry should be considered for the diagnosis of oligodendroglial tumors. In people with oligodendroglial tumors, there is a positive correlation between the degree of biologic malignancy (i.e. tumor grade) and Ki-67 labeling index, and

oligodendrogliomas that exhibit a Ki-67 labeling index >5%, it have been shown to be more refractory to therapy than those with a lower Ki-67 labeling index.[27] Additional veterinary studies will be needed to determine if similar Ki-67 labeling indices have prognostic value in veterinary medicine.

The presence of GFAP positive neoplastic oligodendrocytes has not been previously reported in canine oligodendrogliomas. In humans, GFAP positive cells can be demonstrated in up to 50% of all oligodendrogliomas.[13] This positive staining may be explained by reactive gemistocytic astrocytes trapped within tumor cells. Alternatively, another explanation for positive GFAP immunoreactivity is staining of a minigemistocyte, also called a gliofibrillary oligodendrocyte. These cells are hypothesized to be neoplastic oligodendrocytes transitioning into neoplastic astrocytes.[13] Although there was no statistical significance of GFAP immunoreactivity between tumor types in this study, the application of GFAP positive cells within oligodendrogliomas is another useful tool for the veterinary pathologist. Historically, GFAP immunoreactive cells have been considered as strictly a diagnostic feature of astrocytic tumors. In fact, many veterinary pathologists conventionally use GFAP immunoreactivity as a measure of excluding oligodendroglial tumor diagnoses. Although we did not discriminate between the degree of immunoreactive cells nor the location within the tumor, we consider our findings useful when making future diagnoses of oligodendroglial neoplasms.

The application of EGFR immunocytochemistry to canine oligodendrogliomas in this study had not been previously attempted. We found that EGFR immunoreactivity, although not statistically associated with tumor grade, was indeed present in all tumor grades. Although these findings do not yield additional information for diagnostic criteria of oligodendroglial tumors, the presence of EGFR within various tumors may represent a useful therapeutic strategy for animals with such neoplasms. In humans, EGFR is overexpressed, mutated, or both in many solid tumors.[76] Competitive inhibitors of ATP binding by EGFR include drugs such as gefitinib and erlotinib. These medications are now approved for refractory locally advanced or metastatic non-small-cell lung cancer in humans and are used with variable success. EGFR kinase inhibitors are in phase 1 and 2 testing in other tumor types as well. Approximately 40 percent of glioblastomas in humans show overexpression of EGFR.[77] The responsiveness of EGFR

kinase inhibitors to various CNS tumors in humans remains to be continually investigated. Comparatively, the benefit of EGFR kinase inhibition for canine tumors has not been explored. Our recognition of EGFR expression in this oligodendroglial population lends hope to future investigation of EGFR and other growth factor receptor expression with CNS tumors.

Research into the molecular characterization of human oligodendrogliomas has added vital information regarding prognostic indicators and responses to therapy. Prognostically, the loss of heterozygosity of chromosomes 1p and 19q in humans with oligodendrogliomas is associated with chemotherapy sensitivity and longer recurrence-free survival times. Treatment modalities in humans, as previously mentioned, generally target the tyrosine kinase proteins and their associated growth factor since they have been implicated in tumor progression and transformation. In order to effectively translate similar therapy protocols into canine models, additional investigations to determine if similar molecular changes are present in spontaneous canine tumors is necessary.

In conclusion, we recommend continued grading of spontaneous canine glial neoplasms based on the established WHO classification. In particular, distinguishing canine WHO grade II and III oligodendrogliomas should be based on mitoses and degree of cellular atypia as these were statistically the most useful features. A diagnosis of oligoastrocytoma, or mixed gliomas, should be made based on the identification of two glial cell type populations. Additionally, the recognition of GFAP immunoreactivity should be considered as a diagnostic feature rather than an exclusion criteria of oligodendroglial tumors. EGFR immunoreactivity of variable intensity may also be observed within canine WHO grades II and III oligodendrogliomas and oligoastrocytomas. Lastly, since the median Ki-67 labeling index is significantly associated with tumor grade and lower Ki-67 labeling indices are observed in WHO grade II oligodendrogliomas, we recommend routine application of Ki-67 immunocytochemistry for the diagnosis of oligodendroglial tumors.

Table 1- Signalment, morphologic tumor diagnosis, tumor location, and summary of immunoreactivity in 15 dogs with oligodendrogliomas

Dog	Breed	Age (years)	Sex	Morphological Tumor Diagnosis	Tumor Location	Immunocytochemistry		
						GFAP (-, +)	EGFR (-.+,++, +++)	Ki-67 (%)
1	Bullmastiff	5	FS	Oligodendroglioma (WHO grade II)	Brainstem	-	+	4.14
2	Boston Terrier	11	MN	Oligodendroglioma (WHO grade II)	Right cerebral hemisphere	+	++	1.21
3	Boxer	10	MN	Oligodendroglioma (WHO grade II)	Right cerebral hemisphere	+	+++	3.86
4	Staffordshire Terrier	10	MI	Oligodendroglioma (WHO grade II)	Right cerebral hemisphere	+	+	2.50
5	Miniature Schnauzer	8	FS	Oligodendroglioma (WHO grade II)	Right cerebral hemisphere	-	-	2.50
6	Boxer	3	MN	Oligodendroglioma (WHO grade II)	Left cerebral hemisphere	-	++	3.86
7	English Bulldog	6	MN	Anaplastic oligodendroglioma (WHO grade III)	Right cerebral hemisphere	-	+	10.55
8	Boxer	8	FS	Anaplastic oligodendroglioma (WHO grade III)	Left cerebral hemisphere	+	+	9.34
9	Mixed breed	12	MN	Anaplastic oligodendroglioma (WHO grade III)	Left cerebral hemisphere	-	-	8.96
10	Boston Terrier	12	MI	Anaplastic oligodendroglioma (WHO grade III)	Left cerebral hemisphere	-	++	9.08
11	Boston Terrier	8	MN	Anaplastic oligodendroglioma (WHO grade III)	Left cerebral hemisphere	+	+	9.49
12	Boxer	10	FI	Oligoastrocytoma (WHO grade II)	Left cerebral hemisphere	+	+++	6.46
13	Labrador Retriever	3	MI	Oligoastrocytoma (WHO grade II)	Left cerebral hemisphere	+	+++	6.48
14	Boston Terrier	4	FS	Oligoastrocytoma (WHO grade II)	Brainstem	+	+++	4.71
15	Boston Terrier	12	FS	Oligoastrocytoma (WHO grade II)	Left cerebral hemisphere	+	+++	7.72

Table 2- Results of histomorphologic evaluations and overall associations between histomorphologic variables and types of oligodendroglial tumors in 15 dogs

Histomorphologic Variables	Outcome Measure	Type of oligodendroglial tumor			*P*-value‡
		Oligodendroglioma WHO grade II†	Oligodendroglioma WHO grade III†	Oligoastrocytoma WHO grade II†	
Mitosis	1-5/hpf	6	0	1	0.012*
	6-10/hpf	0	5	3	
Necrosis	Absent	5	3	4	0.714
	Present	1	2	0	
Cytologic Atypia	None	5	0	0	0.002*
	Slight	1	0	2	
	Moderate	0	4	2	
	Marked	0	1	0	
Vascular Hypertrophy	Absent	4	1	3	1.000
	Present	2	4	1	
Vascular Proliferation	Absent	2	1	1	0.156
	Present	3	0	3	
	Present + Marked	1	4	0	

*Statistically significant ($p < 0.05$)

Table 3- Multiple comparisons between histomorphologic variables with significant overall association with tumor type and grade in 15 dogs with oligodendroglial tumors

Tumor Type and Grade Comparison	Histomorphologic Variable							
	Mitoses			Cellular Atypia				
	Outcome comparison		p value	Outcome comparison				p value
	1-5/hpf	6-10/hpf		None	Slight	Moderate	Marked	
Grade III vs.	0	5	0.002*	0	0	4	1	0.004*
Grade II oligo	6	0		5	1	0	0	
Grade II oligo vs.	6	0	0.033	5	1	0	0	0.028
Oligoastrocytoma	1	3		0	2	2	0	
Grade III oligo vs	0	5	0.444	0	0	4	1	0.119
Oligoastrocyoma	1	3		0	2	2	0	

*Statistically significant ($p < 0.0167$) for multiple comparisons
†Numbers are counts (Total n = 15 for each test)

‡Exact *P*-value for association between test outcome and type of oligodendroglial tumor

Figure 1- Summary of Ki-67 Immunoreactivity by Tumor Type

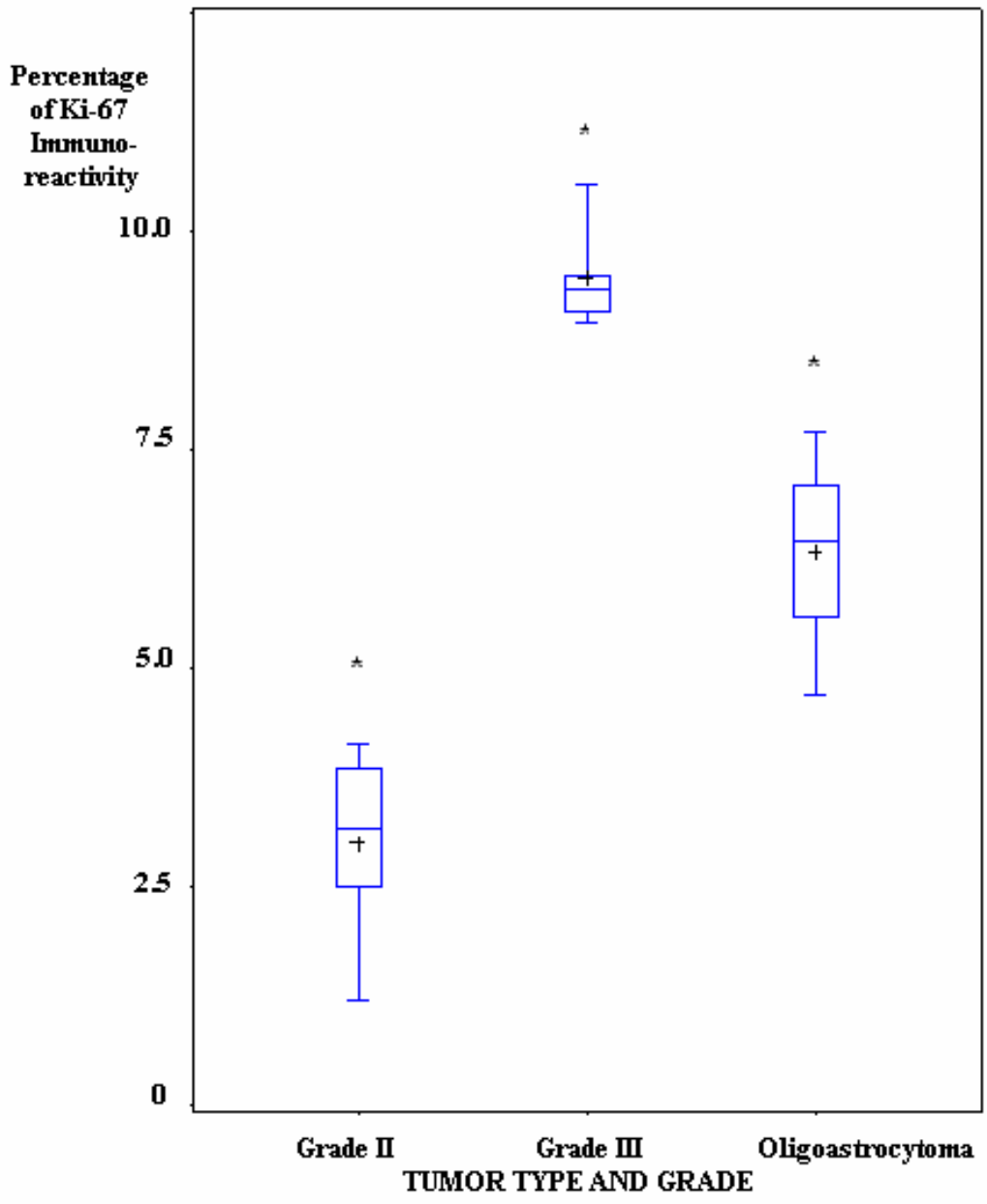


Figure 1 Key

Grade II= WHO Grade II oligodendroglioma

Grade III= WHO Grade III oligodendroglioma

In the plot, boxes represent the interquartile range (IQR), and in each box means are marked with the symbol "+", while medians are represented by horizontal lines through each box**.** The whiskers represent the most extreme observations that are less than 1.5 X IQR from the upper and lower limits of the IQR. The symbol "*" indicates that the median percentage of Ki-67 immunoreactivity of that tumor is significantly different ($p < 0.05$) from the other two tumor types.

Figure 2.1- Canine WHO grade II oligodendroglioma showing clear cytoplasm of tumor cells providing a "fried egg" appearance. The neoplasm has moderate cellularity and has uniform nuclei. (dog no.4, H&E stain, Bar=25μm)

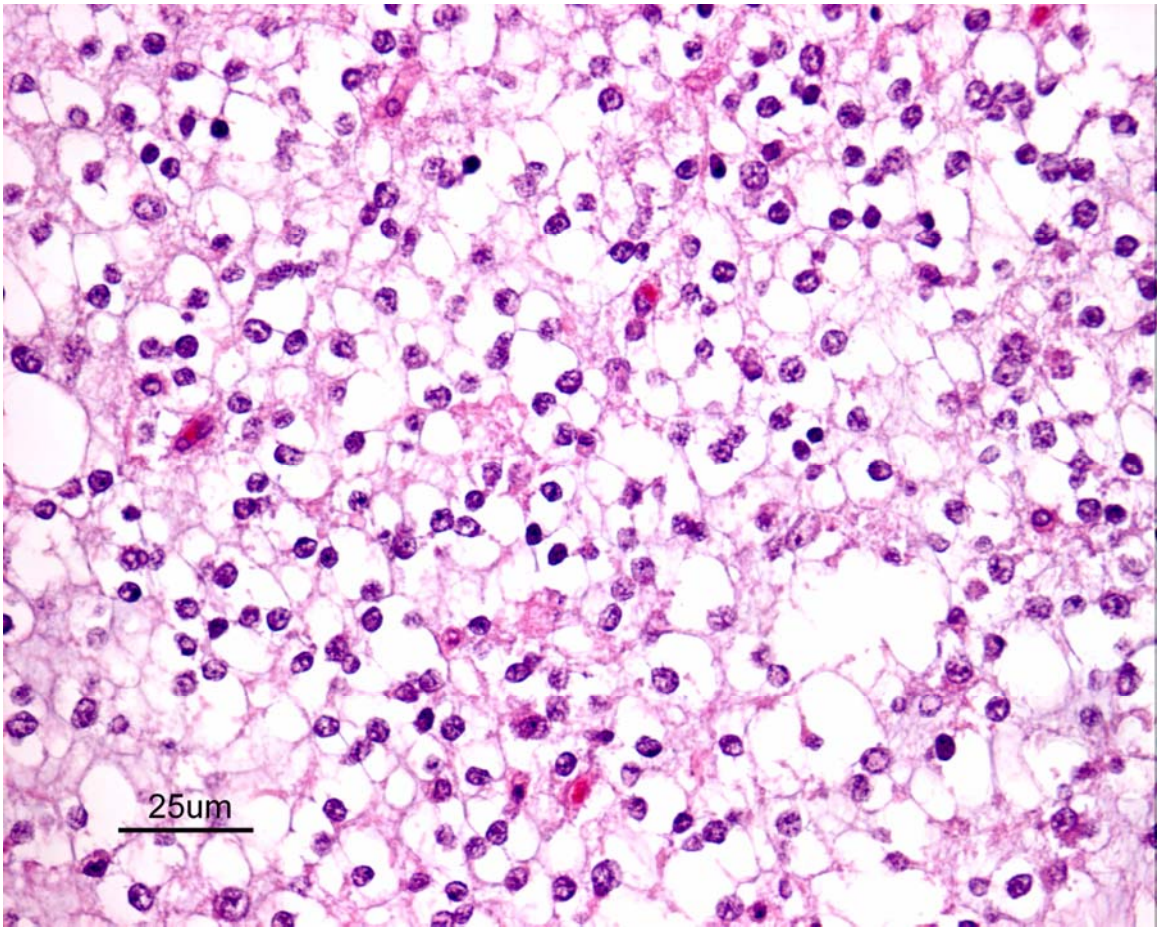


Figure 2.2- Canine WHO grade III oligodendroglioma showing higher cellularity than the WHO grade II oligodendroglioma (Figure 2.1), nuclear polymorphism and mitotic figures (arrow). (dog no.9, H&E stain, Bar=25μm)

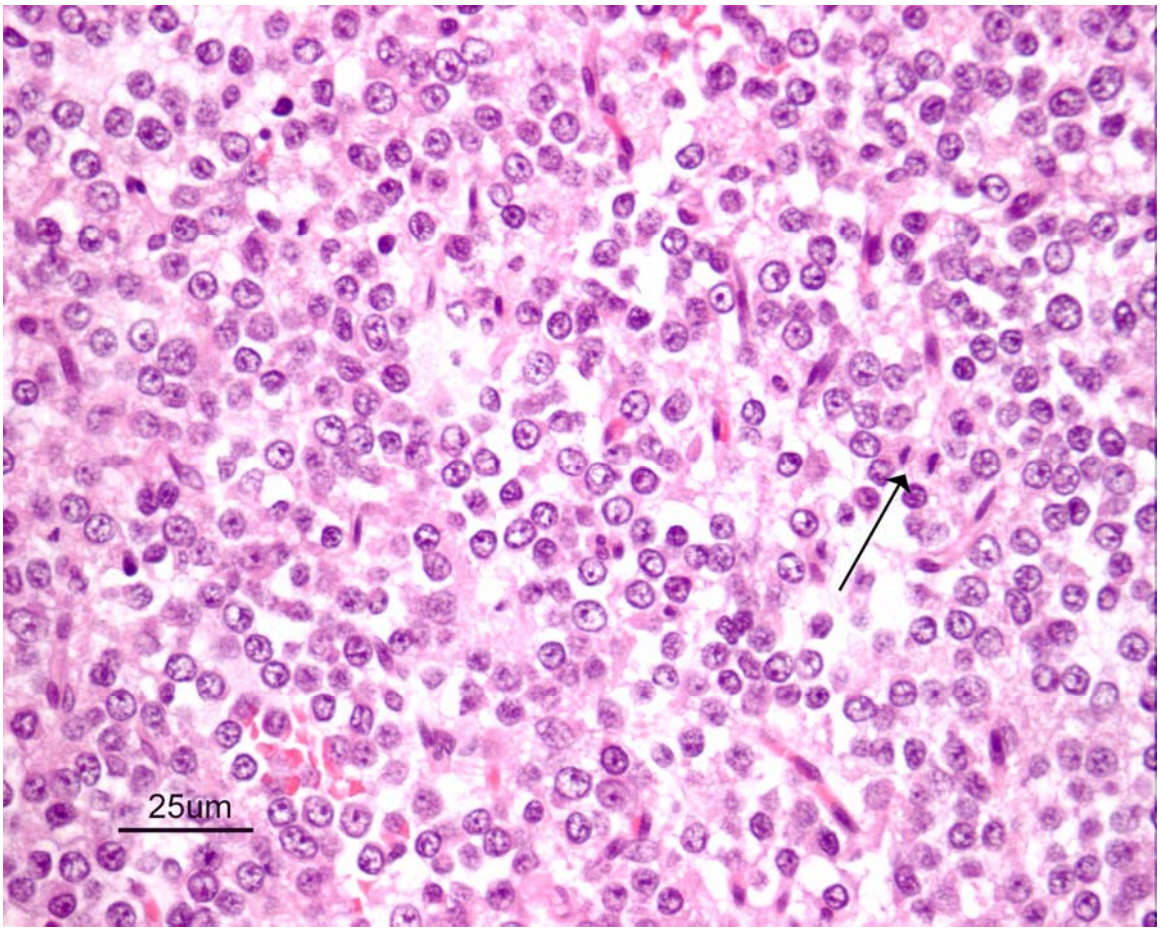


Figure 2.3a- Canine WHO grade II oligoastrocytoma; oligodendroglial component. Numerous neoplastic oligodendrocytes with hyperchromatic nuclei are present along with occasional neoplastic astrocytes (arrow). (dog no.12, H&E stain, Bar=25μm)

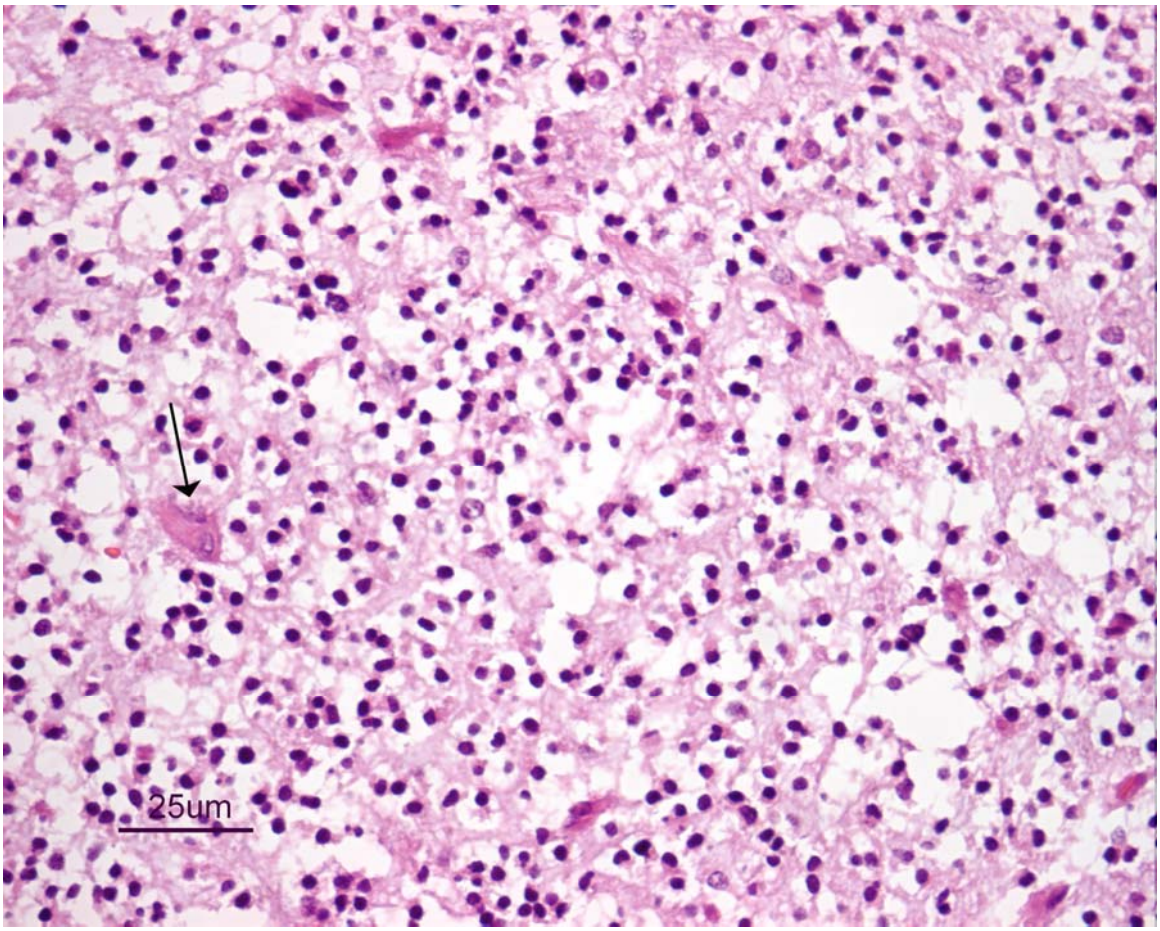


Figure 2.3b- Canine WHO grade II oligoastrocytoma; astrocytic component. This is from the same neoplasm as Figure 2.3a. (dog no.12, H&E stain, Bar=25µm)

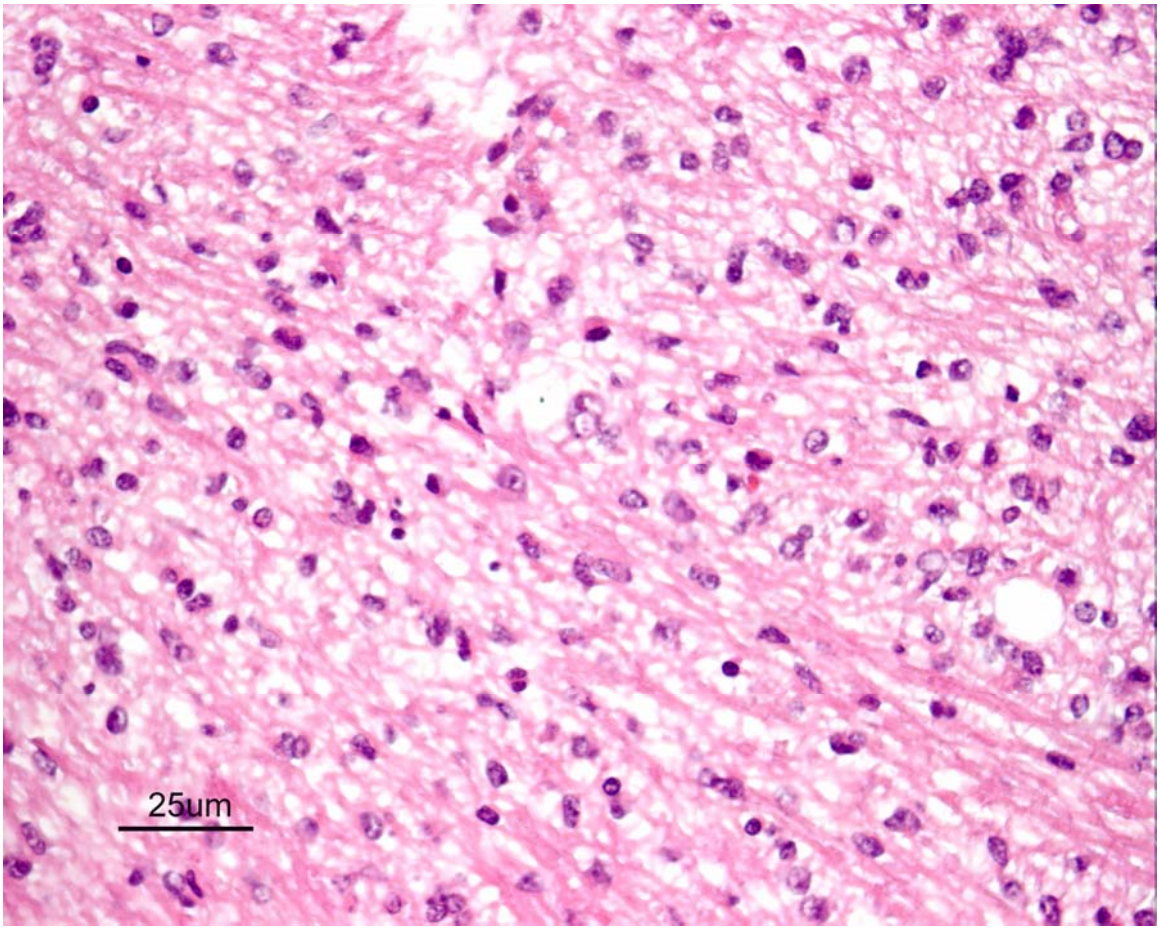


Figure 3a- Vascular proliferation (arrows) in a canine WHO grade III oligodendroglioma (dog no.10, H&E stain, Bar=50μm)

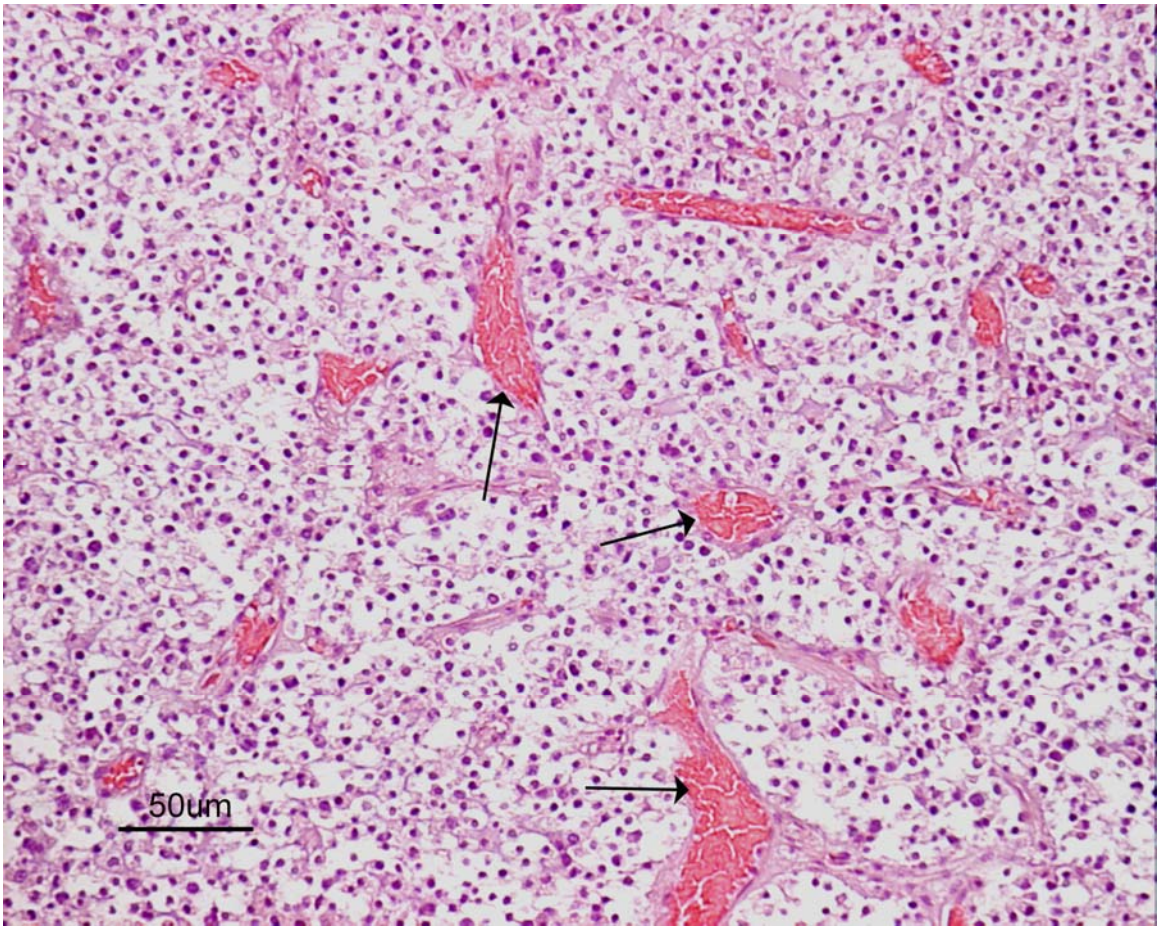


Figure 3b- Vascular hypertrophy in a canine WHO grade III oligodendroglioma. (dog no.10, H&E stain, Bar=25μm)

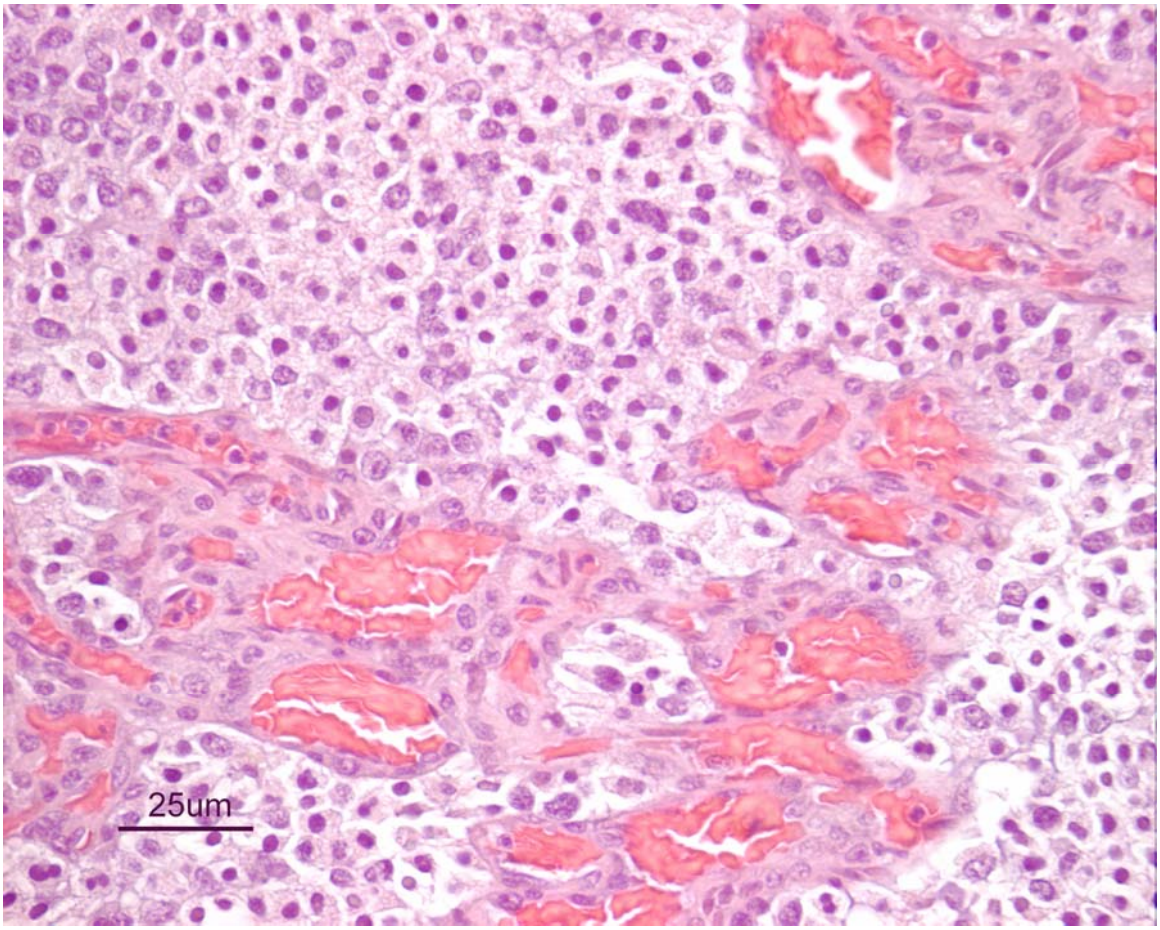


Figure 4a- Extensive region of necrosis (arrows) in a canine WHO grade III oligodendroglioma. (dog no.10, H&E stain, Bar=50μm)

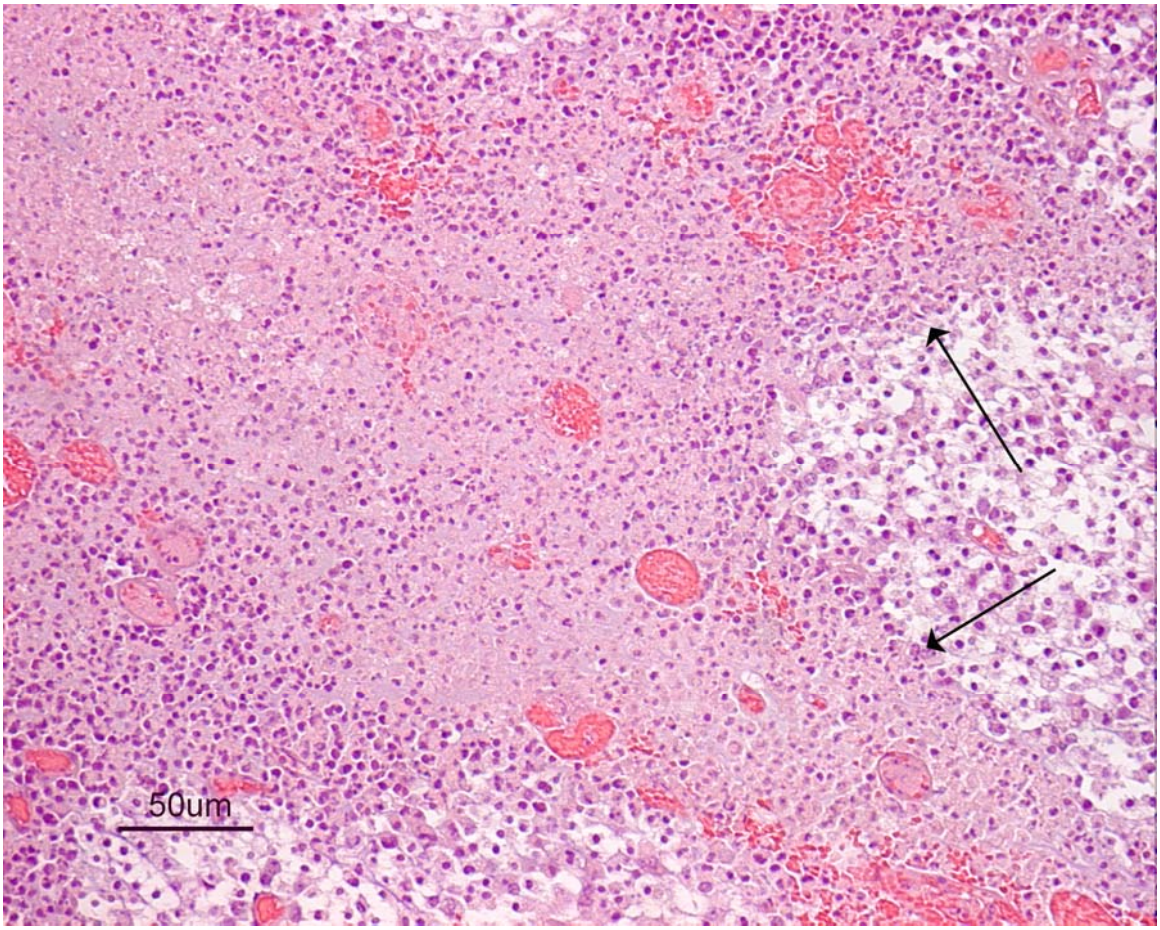


Figure 4b- Necrotic region at higher magnification in a canine WHO grade III oligodendroglioma (dog no.10, H&E stain, Bar= 25µm)

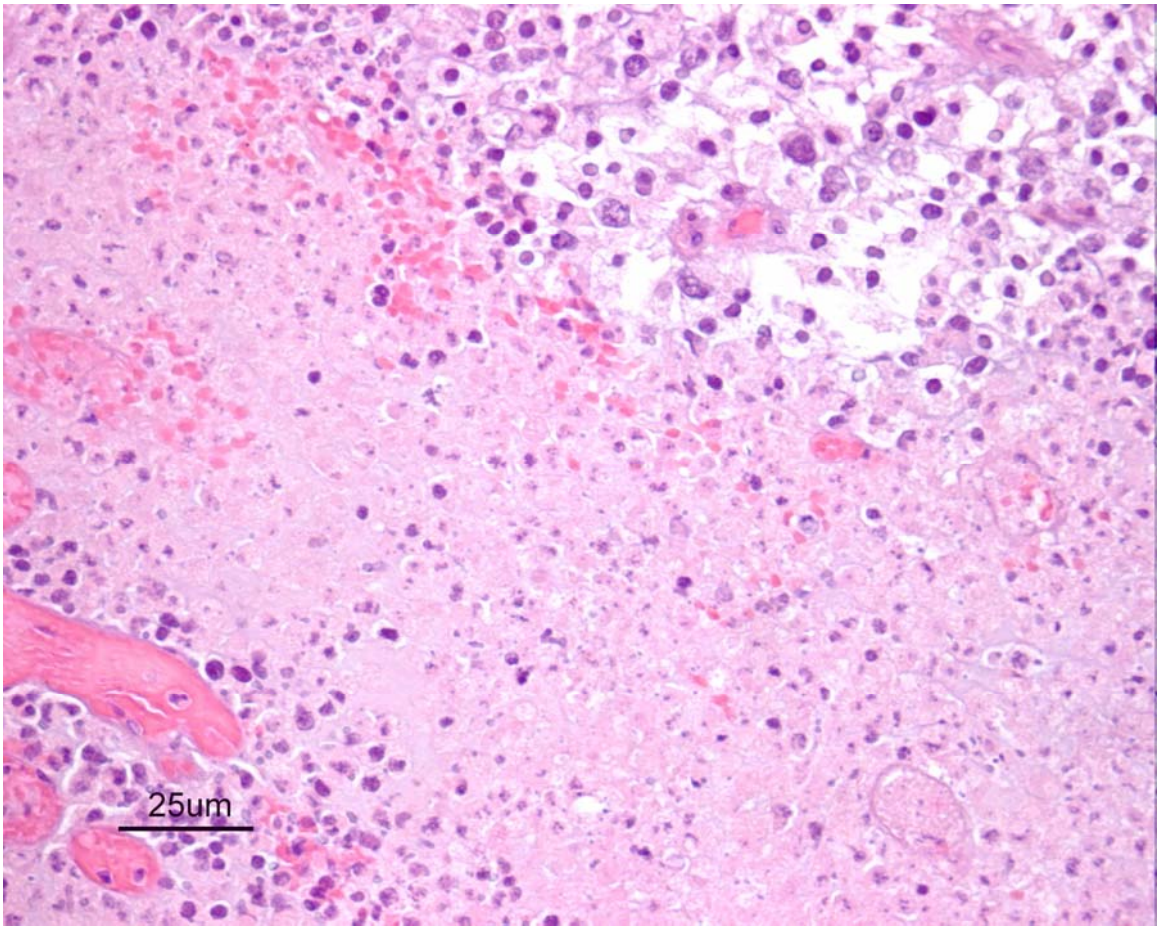


Figure 5a- Mitotic figures (arrow) in a canine WHO grade II oligodendroglioma (dog no.6, H&E stain, Bar=10μm)

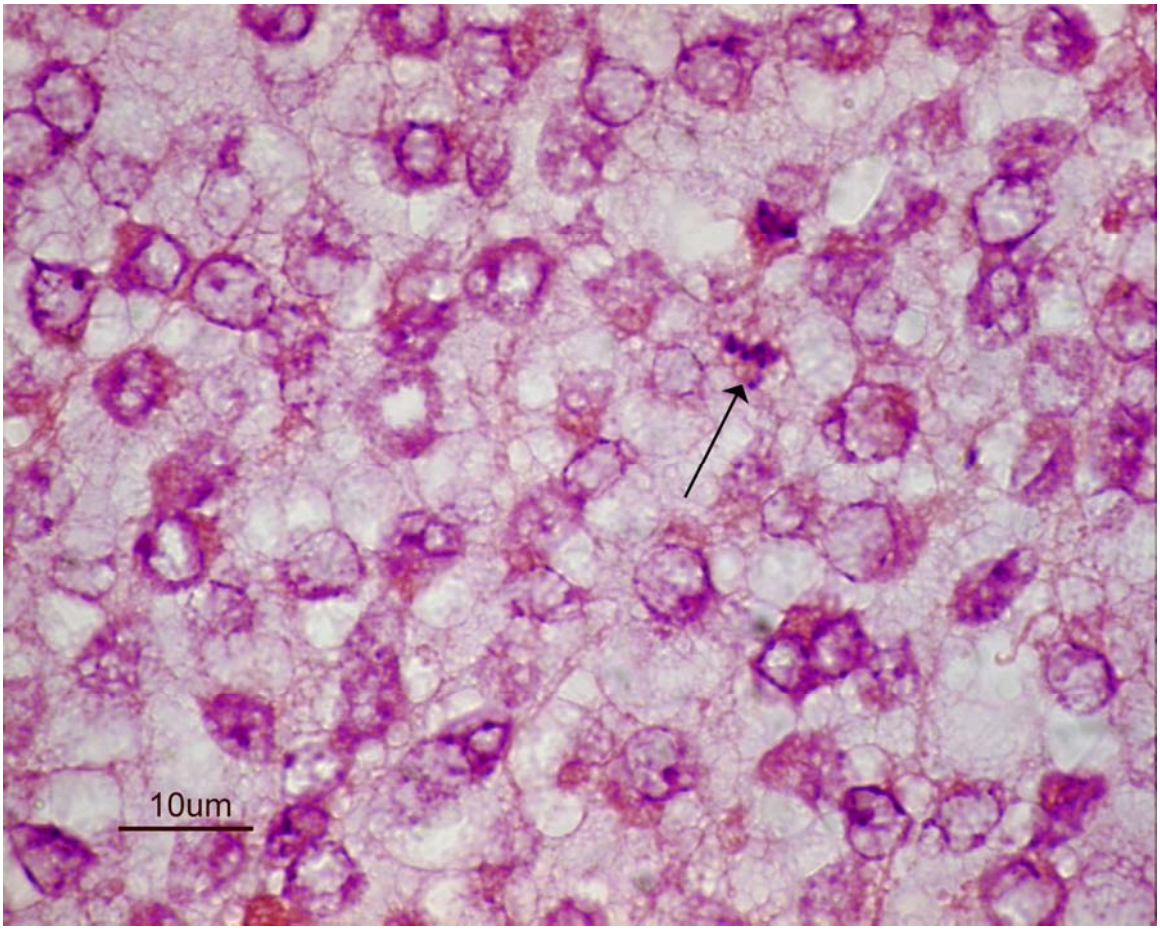


Figure 5b- Mitotic figures (arrows) are readily observed in a canine WHO grade III oligodendroglioma (dog no.9, H&E stain, Bar=10μm)

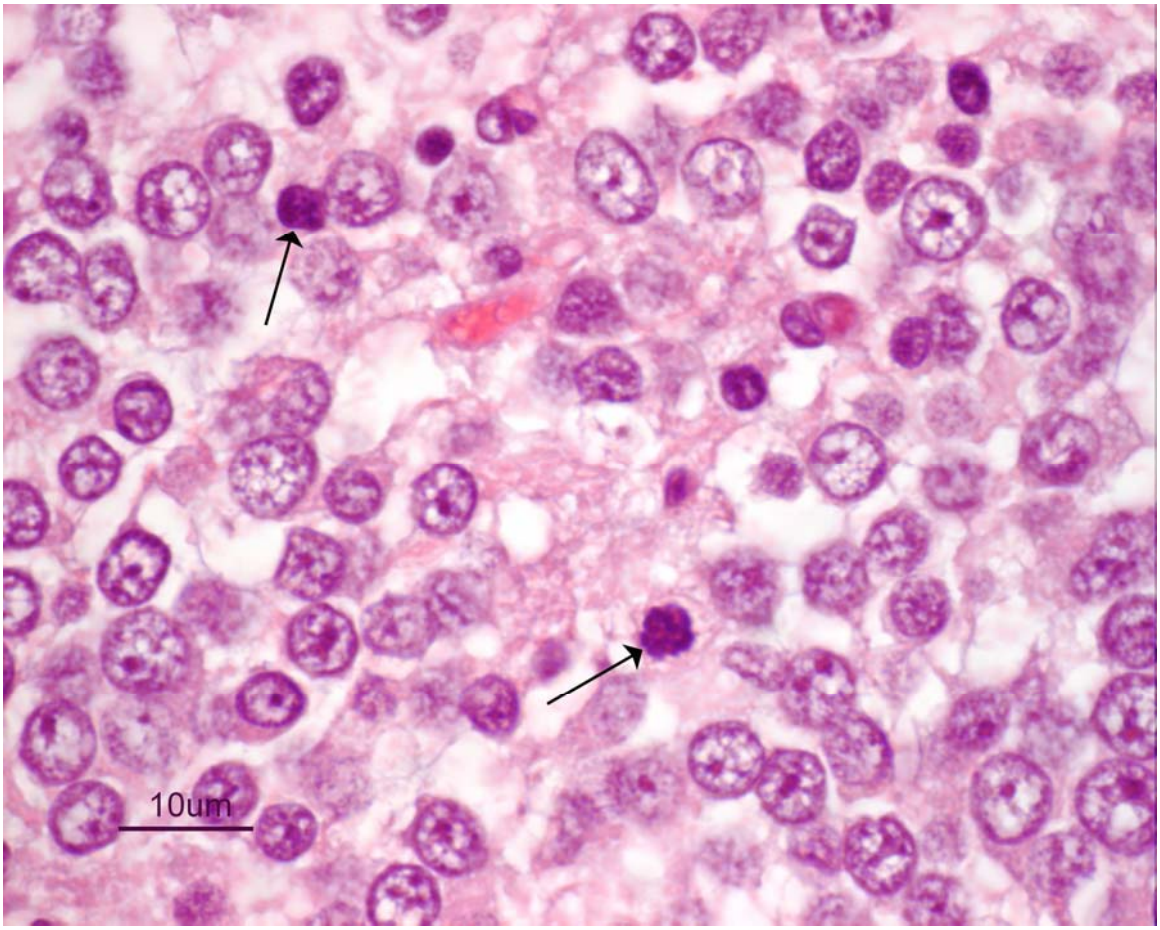


Figure 6a- Overall moderate cellularity with mild cellular atypia observed in a canine WHO grade II oligodendroglioma (dog no.1, H&E stain, Bar=10μm)

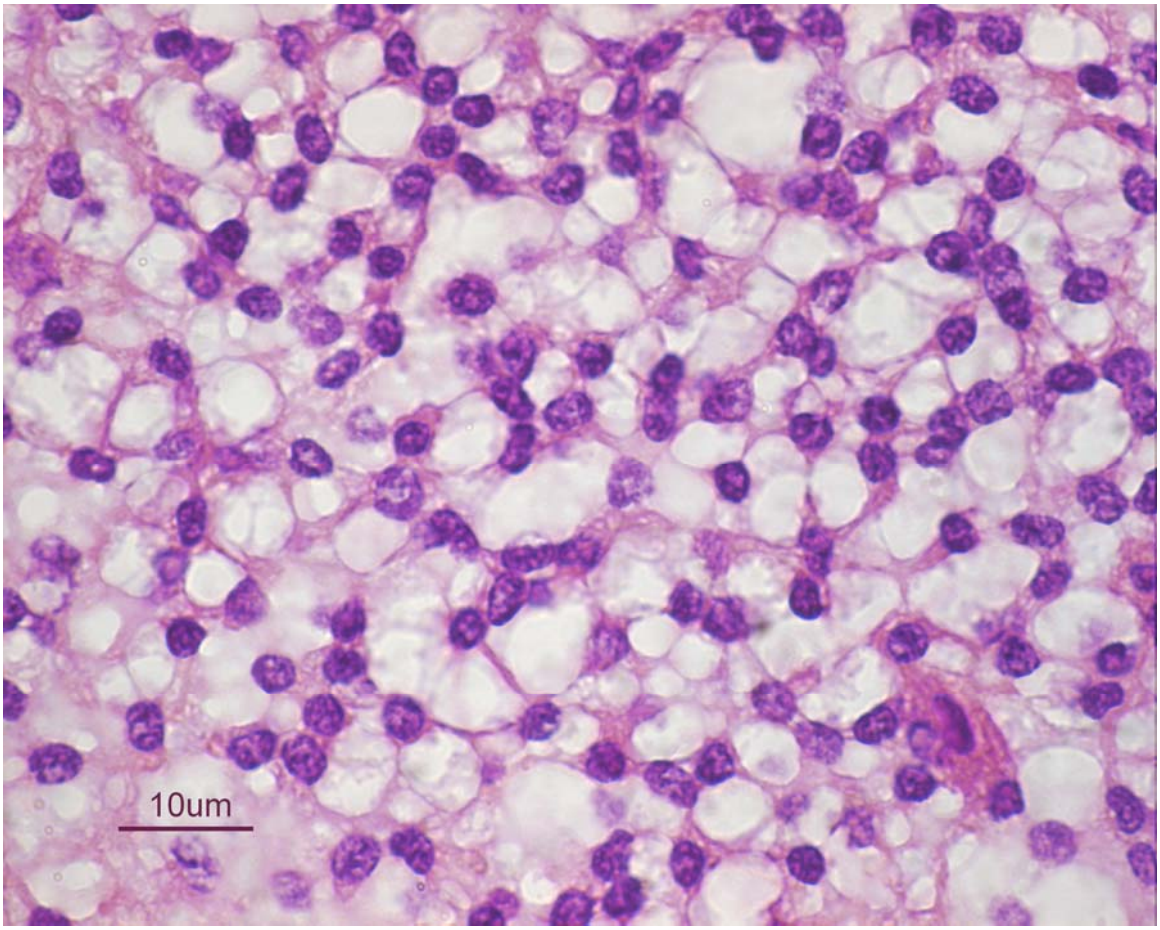


Figure 6b- Marked variation in neoplastic cell size and shape compared to Figure 6a in a canine WHO grade III oligodendroglioma (dog no.10, H&E stain, Bar=10μm)

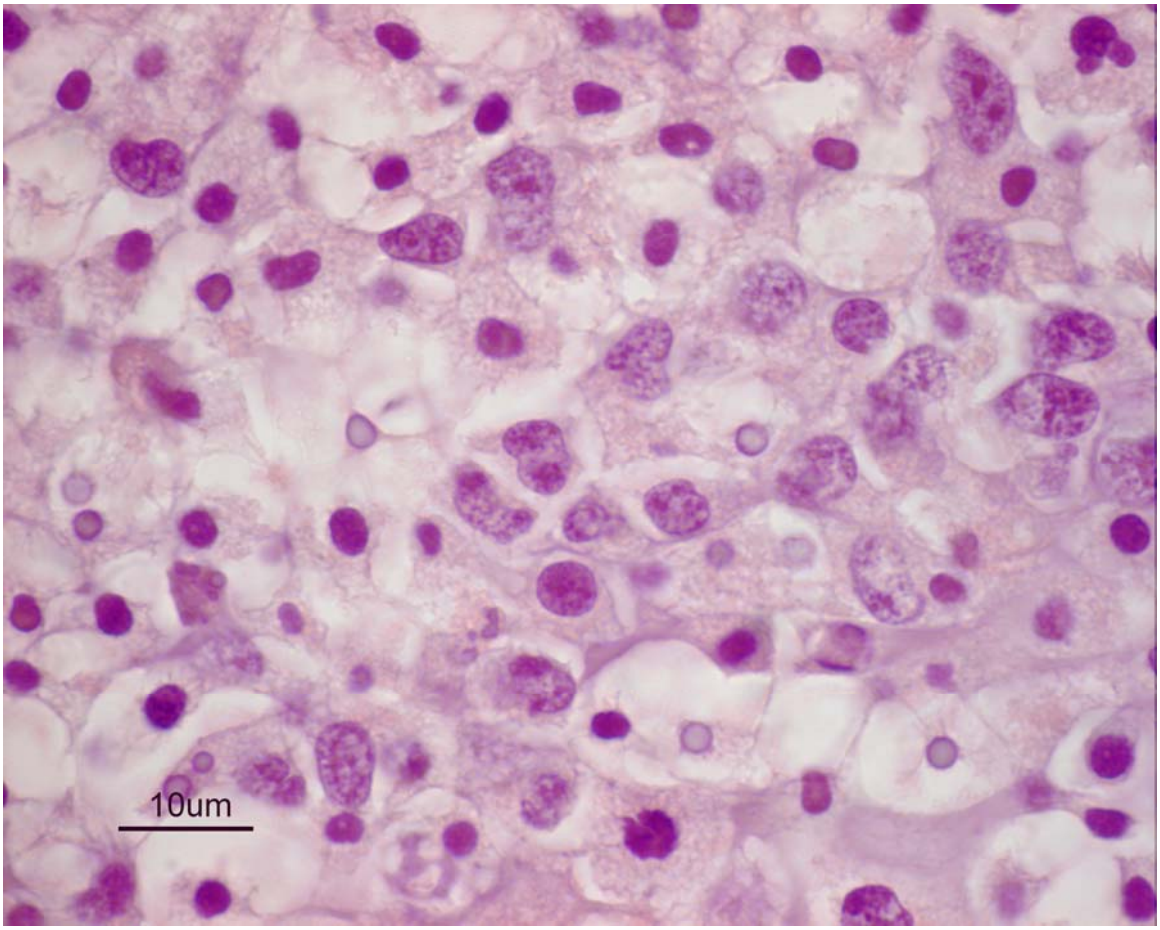


Figure 7a- Red-stained GFAP-positive fibers are noted within the oligodendroglial (A) component of a canine WHO grade II oligoastrocytoma, but are prominent in the astrocytic (B) component (dog no.13, ABC immunoperoxidase with hematoxylin counterstain, Bar=50µm)

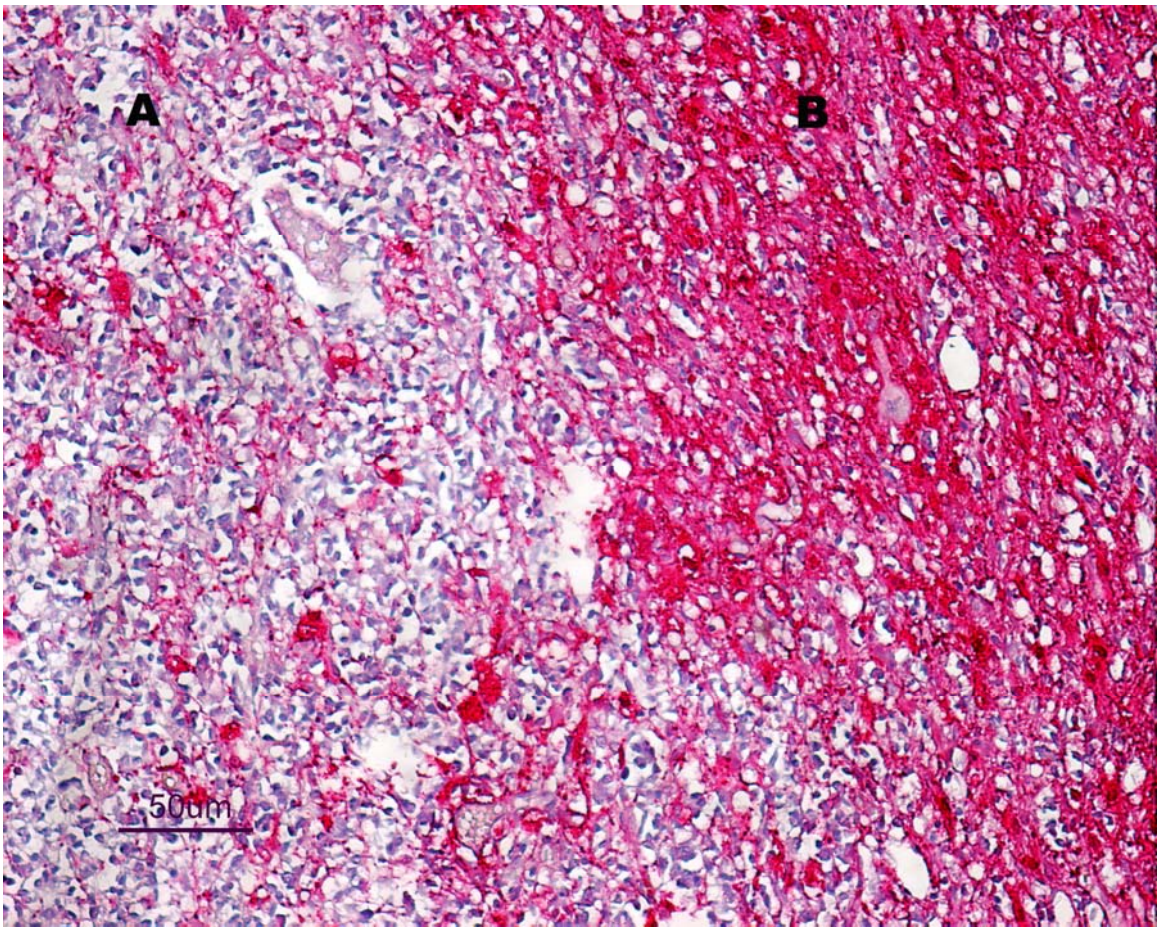


Figure 7b- Scattered red-stained GFAP immunoreactive cells (arrows) noted within a canine WHO grade III oligodendroglioma (dog no.8, ABC immunoperoxidase with hematoxylin counterstain, Bar=50µm)

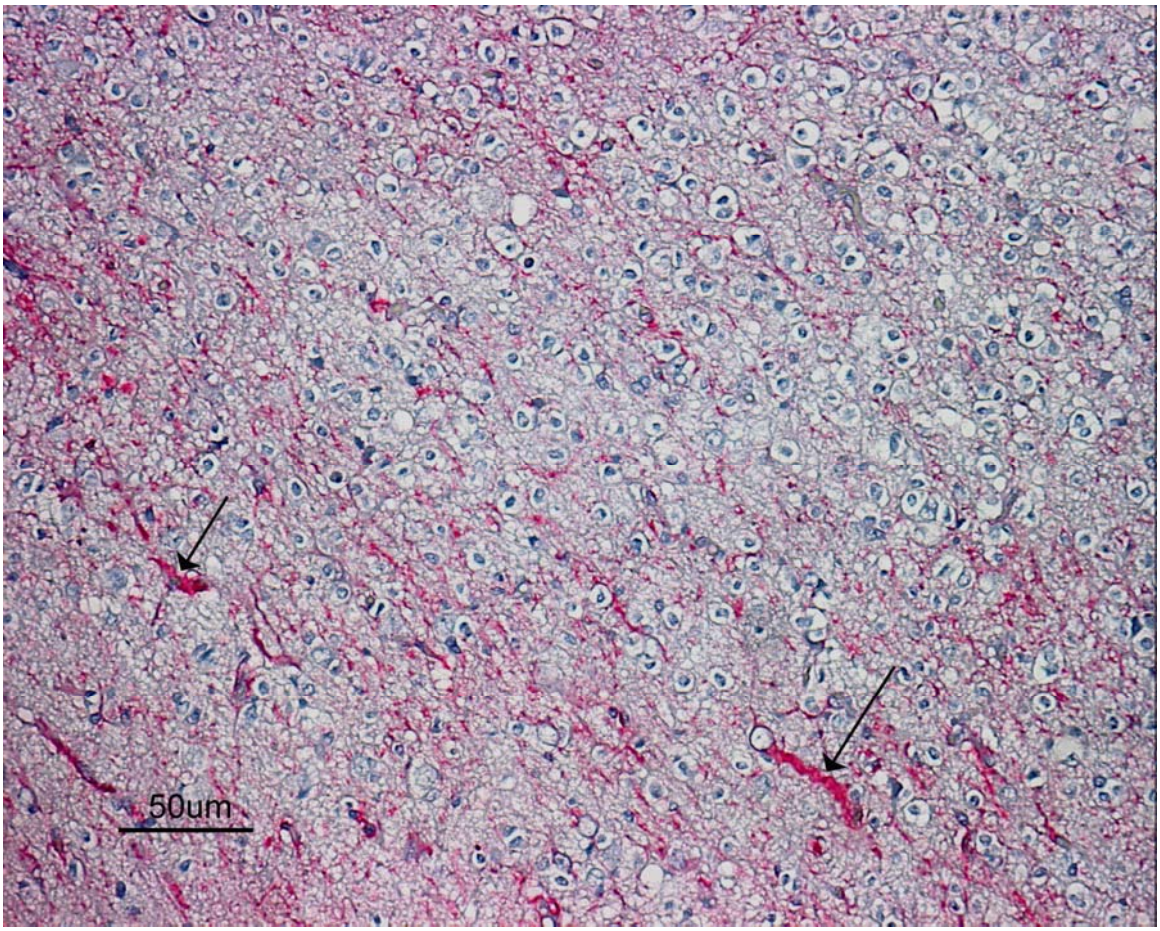


Figure 8a- Mild red-staining EGFR immunoreactive neoplastic cells within a canine WHO grade II oligodendroglioma (dog no.3, ABC immunoperoxidase with hematoxylin counterstain, Bar=10µm)

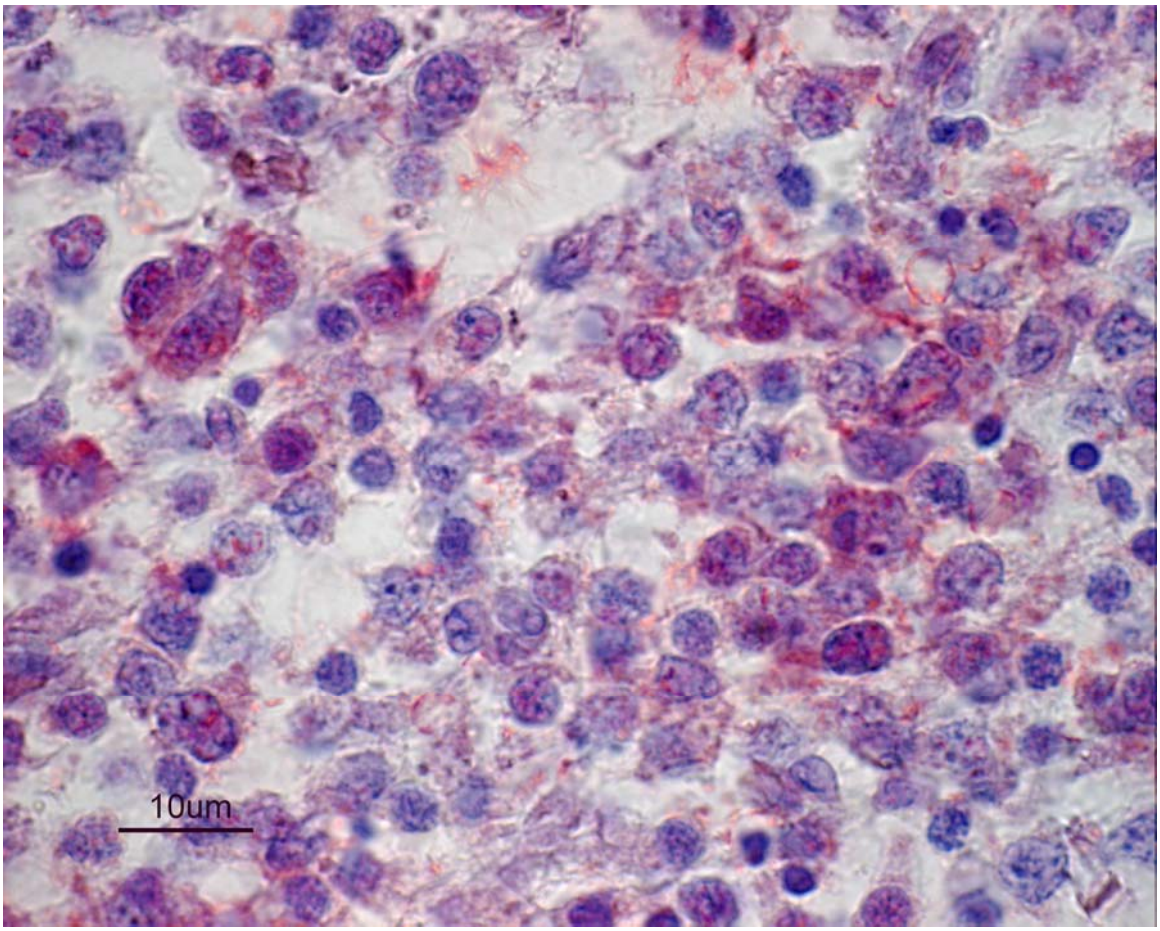


Figure 8b- Many neoplastic cells in a canine WHO grade III oligodendroglioma demonstrate red-staining moderate immunoreactivity for EGFR. (dog no.5, ABC immunoperoxidase with hematoxylin counterstain, Bar=10μm)

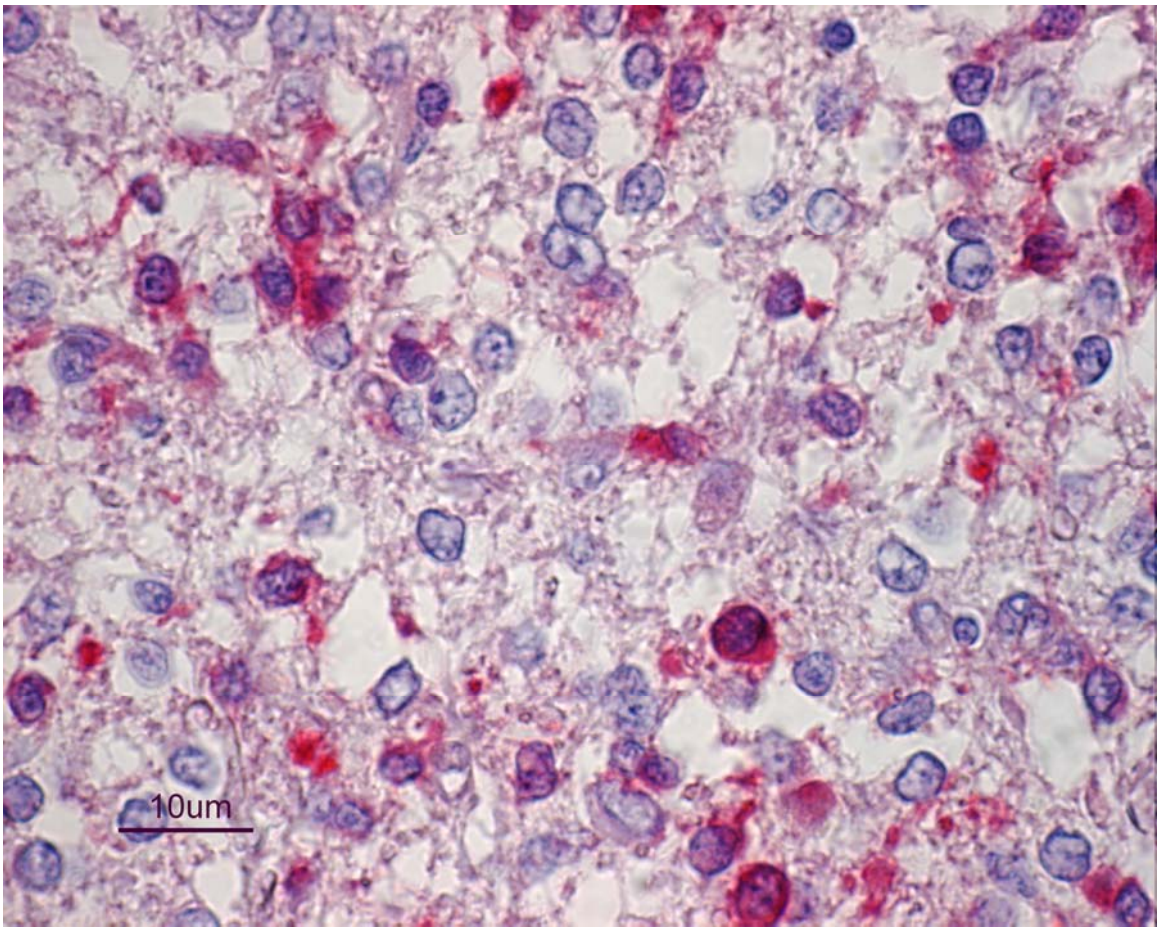
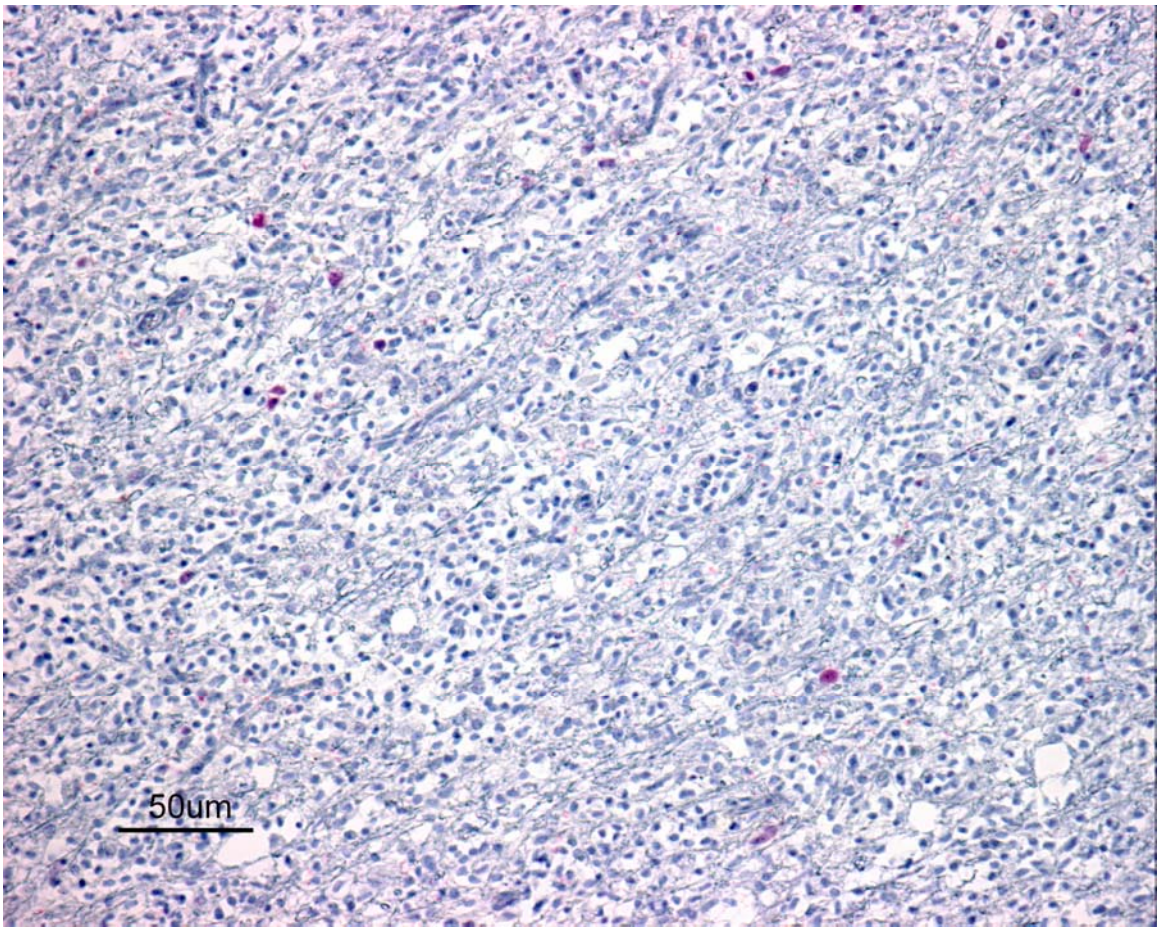


Figure 9a- Red-staining Ki-67 immunoreactive cells with a labeling index of 1.21% in a canine WHO grade II oligodendroglioma (dog no.2, ABC immunoperoxidase with hematoxylin counterstain,



Bar=50μm)

Figure 9b- Red-staining Ki-67 immunoreactive cells with a labeling index of 10.55% in a canine WHO grade III oligodendroglioma (dog no.7, ABC immunoperoxidase with hematoxylin counterstain, Bar=50µm)

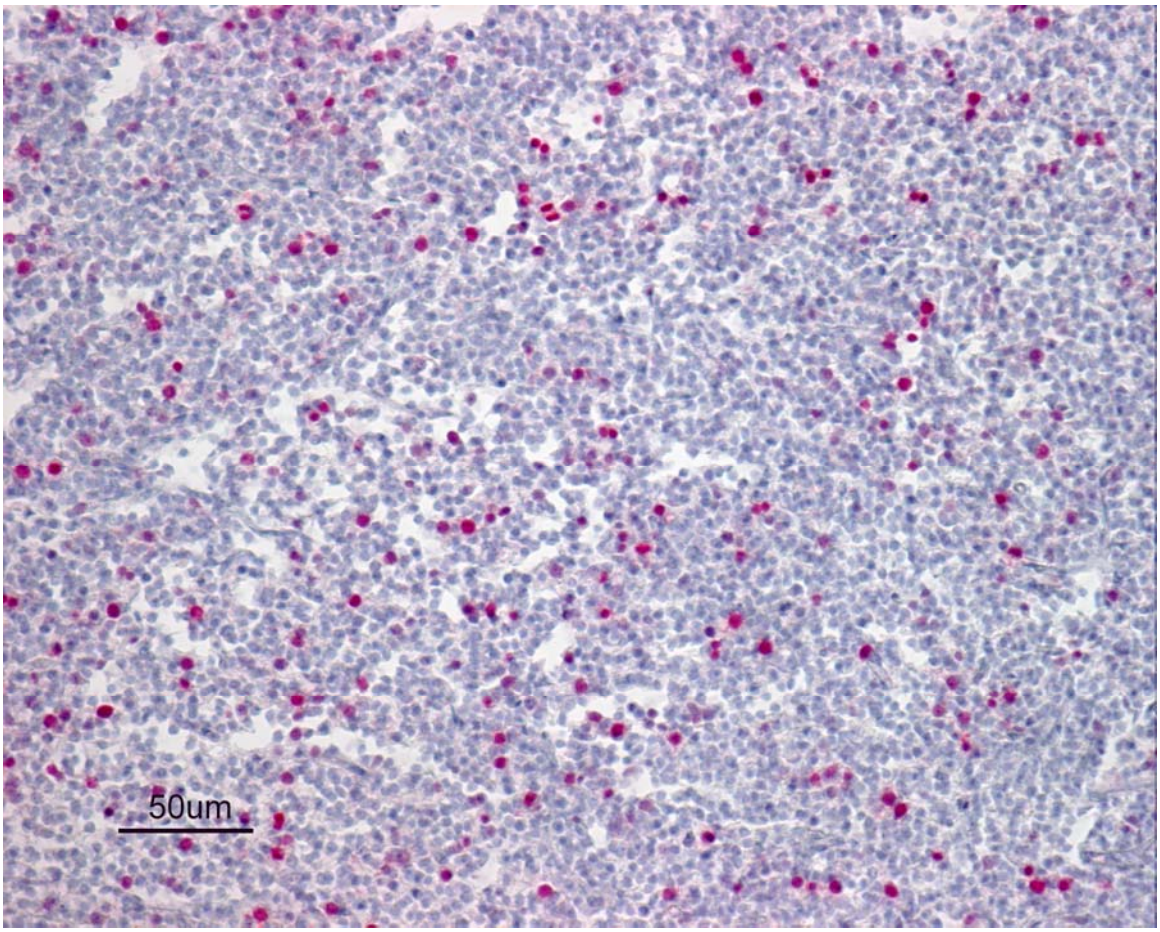
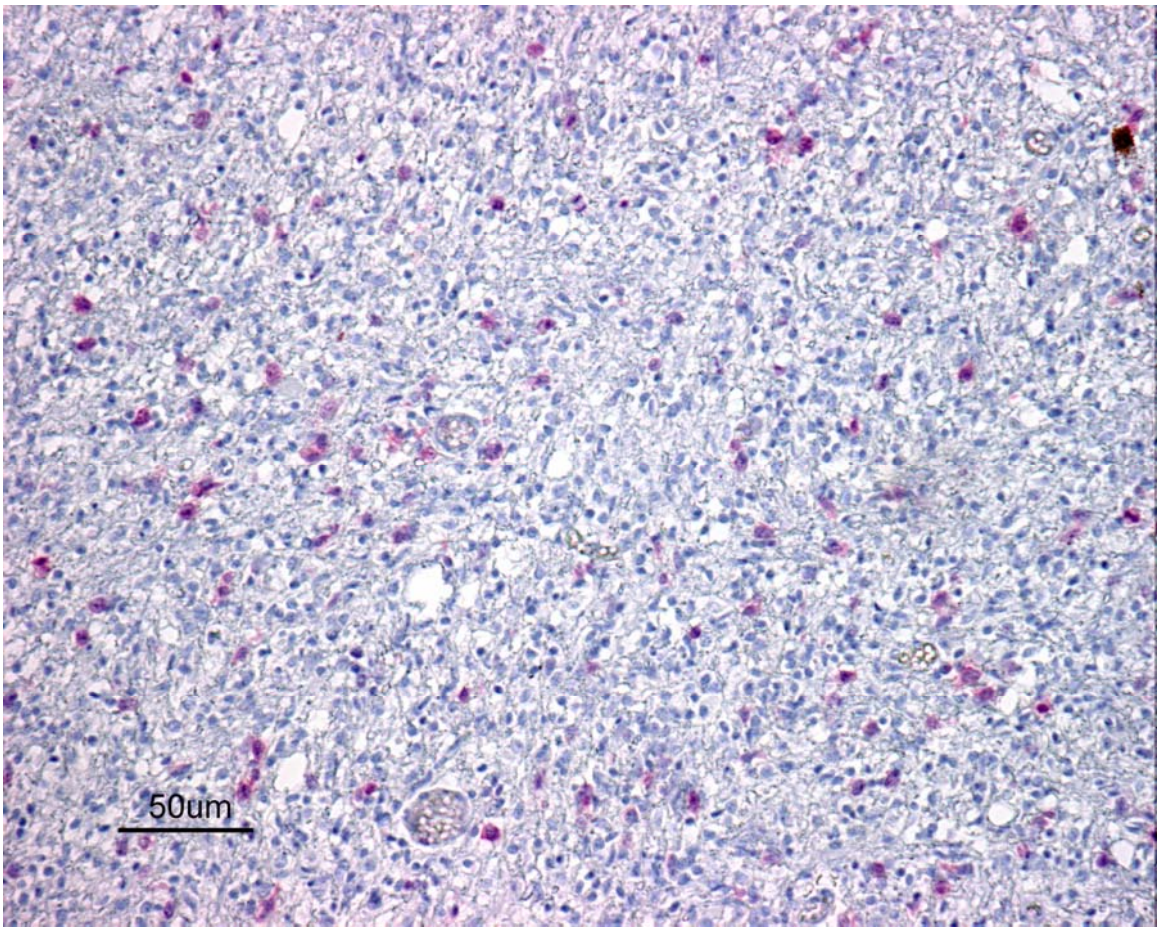


Figure 9c- Red-staining Ki-67 immunoreactive cells with a labeling index of 6.48% in a canine WHO grade II oligoastrocytoma (dog no.13, ABC immunoperoxidase with hematoxylin counterstain, Bar=50µm)



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Appendix

Results of Immunocytochemical Investigations for Non-Significant Variables

Table 1.1- Overall association between GFAP immunoreactivity and type of oligodendroglial tumor in 15 dogs

	Score	**Type of oligodendroglial tumor**			***P*-value‡**
		Oligodendroglioma WHO grade II†	Anaplastic Oligodendroglioma WHO grade III†	Oligoastrocytoma WHO grade II†	
GFAP Immunoreactivity	Absent	3	3	0	0.2138
	Present	3	2	4	

†Numbers are counts of individual cases of tumors (total n=15)
‡Exact *P*-value for association between GFAP immunoreactivity and grade of oligodendroglial tumor.

Tables 1.2-1.4- Associations of GFAP immunoreactivity between grades of oligodendroglial tumor in 15 dogs (performed post hoc)

Table 1.2- Comparison of WHO grade II and WHO grade III oligodendrogliomas

	Score	**Type of oligodendroglial tumor**		***P*-value‡**
		Oligodendroglioma WHO grade II†	Anaplastic Oligodendroglioma WHO grade III†	
GFAP Immunoreactivity	Absent	3	3	0.4329
	Present	3	2	

†Numbers are counts of individual cases of tumors (total n=11)
‡Exact *P*-value for association between GFAP immunoreactivity and grade of oligodendroglial tumor.

Table 1.3- Comparison of WHO grade II oligodendrogliomas and WHO grade II oligoastrocytomas

	Score	**Type of oligodendroglial tumor**		***P*-value‡**
		Oligodendroglioma WHO grade II†	Oligoastrocytoma WHO grade II†	
GFAP Immunoreactivity	Absent	3	0	0.4079
	Present	3	4	

†Numbers are counts of individual cases of tumors (total n=10)
‡Exact *P*-value for association between GFAP immunoreactivity and grade of oligodendroglial tumor.

Table 1.4- Comparison of WHO grade III oligodendrogliomas and WHO grade II oligoastrocytomas

	Score	**Type of oligodendroglial tumor**		***P*-value‡**
		Oligodendroglioma WHO grade III†	Oligoastrocytoma WHO grade II†	
GFAP Immunoreactivity	Absent	3	0	0.1190
	Present	2	4	

†Numbers are counts of individual cases of tumors (total n=9)
‡Exact *P*-value for association between GFAP immunoreactivity and grade of oligodendroglial tumor.

Table 1.5- Overall association between EGFR immunoreactivity and type of oligodendroglial tumor in 15 dogs

	Score	Type of oligodendroglial tumor			*P*-value‡
		Oligodendroglioma WHO grade II†	Anaplastic Oligodendroglioma WHO grade III†	Oligoastrocytoma WHO grade II†	
EGFR Immunoreactivity	none	1	1	0	0.0788
	Mild	2	3	0	
	Moderate	2	1	0	
	Severe	1	0	4	

†Numbers are counts of individual cases of tumors (total n=15)
‡Exact *P*-value for association between EGFR immunoreactivity and grade of oligodendroglial tumor.

Tables 1.6-1.8- Associations of EGFR immunoreactivity between grades of oligodendroglial tumor in 15 dogs (performed post hoc)

Table 1.6- Comparison of EGFR immunoreactivity between WHO grade II and WHO grade III oligodendrogliomas

	Score	**Type of oligodendroglial tumor**		***P*-value‡**
		Oligodendroglioma WHO grade II†	Anaplastic Oligodendroglioma WHO grade III†	
EGFR Immunoreactivity	None	1	1	0.5260
	Mild	2	3	
	Moderate	2	1	
	Severe	1	0	

†Numbers are counts of individual cases of tumors (total n=11)
‡Exact *P*-value for association between EGFR immunoreactivity and grade of oligodendroglial tumor.

Table 1.7- Comparison of EGFR immunoreactivity between WHO grade II oligodendrogliomas and WHO grade II oligoastrocytomas

	Score	**Type of oligodendroglial tumor**		***P*-value‡**
		Oligodendroglioma WHO grade II†	Oligoastrocytoma WHO grade II†	
EGFR Immunoreactivity	None	1	0	0.0333
	Mild	2	0	
	Moderate	2	0	
	Severe	1	4	

†Numbers are counts of individual cases of tumors (total n=10)
‡Exact *P*-value for association between EGFR immunoreactivity and grade of oligodendroglial tumor.

Table 1.8- Comparison of EGFR immunoreactivity between WHO grade III oligodendrogliomas and WHO grade II oligoastrocytomas

	Score	**Type of oligodendroglial tumor**		***P*-value‡**
		Oligodendroglioma WHO grade III†	Oligoastrocytoma WHO grade II†	
EGFR Immunoreactivity	none	1	0	0.0159
	Mild	3	0	
	Moderate	1	0	
	Severe	0	4	

†Numbers are counts of individual cases of tumors (total n=9)
‡Exact *P*-value for association between EGFR immunoreactivity and grade of oligodendroglial tumor.

Vita

Michael was born on November 14, 1975 in Biloxi, Mississippi. He grew up in Chantilly, Virginia and graduated from Virginia Tech with a B.S. in Animal Science in 1997. Subsequently, Michael attended Ross University School of Veterinary Medicine in St. Kitts, West Indies and completed his clinical year at the North Carolina State University College of Veterinary Medicine. After receiving his D.V.M. in 2002, Michael was accepted into a small animal rotating internship at Auburn University. He returned to Blacksburg, Virginia in 2003 to begin a small animal neurology residency at the Virginia-Maryland Regional College of Veterinary Medicine. He also pursued a Master of Science degree in Biomedical and Veterinary Sciences. Michael completed his residency in July 2006 and will be relocating to Vancouver, British Columbia to work with the Canada West Veterinary Specialists and Critical Care Hospital.