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Review Article

The role of regulatory T cells in central nervous system diseases in dogs: fighting with a double-edged sword toward translational discoveries

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Abstract: Regulatory T cells (Tregs) are a recently discovered subpopulation of immunosuppressive T cells critical for sustaining immune homeostasis and self-tolerance. Depending on the disease, Tregs can alter immune responses by exerting their effects on various immunological functions. Evidence reveals that altered Treg homeostasis plays an important role in the development and progression of several central nervous system (CNS) diseases in humans. There have been positive outcomes in trials aimed at restoring Treg balance, but their clinical success has been limited. This could partly be due to a lack of an animal model to recapitulate the complex immune alterations that occur as the disease progresses. Therefore, there is a need for naturally occurring, immune-competent disease models to serve as the next critical step in translating therapy from murine models to human clinical trials. Several CNS diseases have canine analogs due to high inter-species homology of molecular signatures and clinicopathologic features. Human multiple sclerosis, Alzheimer's disease, glioblastoma, and meningioma parallel canine granulomatous meningoencephalomyelitis (GME), canine cognitive dysfunction (CCD), high-grade glioma (HGG), and canine meningioma, respectively. In addition, pet dogs share the same living environment and therefore may offer a compelling model faithfully recapitulating these diseases. This review discusses the current knowledge of Treg homeostasis and dysregulation mechanisms within canine CNS diseases, their analogous diseases in humans, and the ongoing clinical trials aimed at restoring normal Treg function. Collectively, comparative translational research investigating Treg homeostasis is needed to bridge well-designed pre-clinical canine studies with early-phase human clinical trials, ultimately benefiting the health of both species.

Keywords: Regulatory T cell, dogs, canine cognitive dysfunction, Alzheimer's disease, glioma, MS, GME

Introduction

Within the last century, there has been a steadily growing burden of neurological diseases in the human population [1]. With improvements in healthcare and lifestyle, our lifespans have been markedly extended, thus unmasking central nervous system (CNS) decline associated with advanced age, and consequently increasing the prevalence of CNS diseases and neurodegenerative disorders such as glioblastoma (GBM) and Alzheimer's disease (AD) [1, 2]. At the same time, not all neurological burdens are associated with aging. Diseases such as multiple sclerosis (MS) and certain brain tumors are similarly being diagnosed more frequently [2, 3]. Although the pathophysiology varies markedly across the mentioned diseases, an abnormal or altered immune homeostasis shifting towards either sustained neuroinflammation or sustained immunosuppression has emerged as a common link and is considered an important

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contributor to the development and progression of these CNS diseases [4-8]. The previous dogma of the CNS being immune-privileged has been challenged, and current knowledge suggests that immune cells constantly survey the CNS with regulations set by the blood-brain barrier (BBB) and resident glial cells [5]. Collectively, this has provided a rationale for the study of the immunology of CNS disease and led to a large volume of research.

Regulatory T cells (Tregs), first discovered in 1995 by Sakaguchi et al., are considered a subpopulation of immunosuppressive T cells critical for sustaining immune homeostasis and self-tolerance [9]. They are characterized by the expression of CD4 and CD25 (interleukin 2 receptor alpha chain) and the presence of intranuclear forkhead box P3 (FOXP3) transcription factor, which is responsible for the phenotype and function of these cells [9, 10]. Tregs achieve immunosuppression by exerting their effects on effector T cells, natural killer cells, B cells, and antigen-presenting cells. Their dysfunction or imbalances in homeostasis can result in autoimmunity or immunosuppression, which ultimately may lead to the development and progression of multiple CNS diseases [8, 11]. For instance, a reduced number of Tregs, expression of their markers, and associated cytokines have been observed in patients with multiple sclerosis and are associated with autoimmunity [8, 11]. Quantitative and qualitative changes in peripheral Tregs have been implicated in the inflammaging of individuals affected with AD [8, 11]. In glioblastoma, accumulation of Tregs within the tumor microenvironment results in local immunosuppression, leading to tumor progression and damage to the health of surrounding tissues [8, 11]. Increased peritumoral and systemic Treg presence in glioblastoma was correlated to multidimensional immunosuppression and decreased patient survival [12-14], and the central role Tregs play in glioblastoma has driven scientists to propose immunotherapeutic methods to combat the disease [15-20]. Human clinical trials aimed at impaired Tregs explored a variety of immunomodulatory drugs and vehicles to protect Treg populations and functions [21-28]. Clinical trials for glioblastoma and meningioma have sought to inhibit the function of Tregs within the tumor microenvironment through immunomodulation, peptide-based vaccines, monoclonal antibodies, signaling pathway inhibitors, and chemotherapeutic drugs [21-28]. Although some trials reported positive outcomes, most had limited success, highlighting the potential for targeting Tregs but also the need to optimize the development of therapeutic strategies [15-28].

Murine models have proved invaluable for preclinical research. However, the literature suggests limitations in their translational value, leading to limited clinical successes for human patients. They are typically genetically homogeneous and possess an impaired immune systems with minimal exposure to environmental antigens [29-33]. Naturally occurring disease models that faithfully recapitulate complex immune responses (including those mediated by Tregs) might empower the translation of preclinical data generated using murine models to human trials. Domesticated mammals, like dogs, harbor naturally occurring diseases to facilitate translation. The strengthening human-animal bond places dogs within the same living environment and thereby exposes them to the same neurotoxins as their human counterparts [29, 30, 34]. Due to highly conserved molecular signatures and clinicopathologic features, diseases such as MS, AD, glioblastoma, and meningioma have naturally occurring canine analogs - canine granulomatous meningoencephalomyelitis (GME), canine cognitive dysfunction (CCD), canine high-grade glioma (HGG), and canine meningioma, respectively [29]. Comparative, translational research investigating Treg homeostasis across both species may allow fast-track clinical investigations that benefit both species.

This review discusses the current understanding of alterations in Treg homeostasis for several canine CNS diseases in dogs that have high translational value. We explore the pathways associated with Treg function, summarize the mechanisms of dichotomous Treg responses in different disease contexts across both species, and review the results of ongoing clinical trials aiming to alter Treg homeostasis or function. Lastly, we outline areas for which more validation in dogs is critically needed.

Regulatory T cells

Regulatory T cells (Tregs) are a subset of T cells whose primary function is maintaining self-tol-

erance and immune homeostasis by immunosuppression [9]. In general, FOXP3$^+$ Tregs can be categorized into thymus-derived and peripherally induced Treg cell subsets. Of note, several other Treg subsets have been reported in the literature (i.e., Tr1, Th3, or iTreg35) [35]. However, the role that these latter subsets play in the pathology of canine health or disease is not well-defined currently [30]. The defining characteristics of human Tregs include high expression of CD25, a subunit of the IL-2 receptor, and intranuclear forkhead box P3 (FOXP3) transcription factor, low IL7 (CD127) and/or glucocorticoid-induced tumor necrosis factor (GITR) presence [36]. The mutual presence of high CD25 expression and FOXP3 is considered a Treg-specific marker in human patients, despite transiently elevated levels of CD25 and minimal presence of FOXP3 being reported in other cell subtypes, such as activated non-suppressive CD4$^+$ T cells, B cells, and macrophages [37, 38].

Like their human counterparts, canine Tregs are identified by expression of CD4, high CD25, and FOXP3 [29, 30]. Canine Tregs show a conservation of suppressive effects on the proliferation of CD4$^+$, CD25$^-$ T lymphocytes similar to human and murine Tregs, further revealing functional similarities across the species barriers [29, 30]. To determine the extent of genomic likenesses between human, murine, and canine Tregs, transcriptomic signatures were evaluated in each species [29, 30]. The majority of the Treg-specific transcripts and expression profiles documented in human Tregs have also been revealed in canine Tregs, including but not limited to IL-10, FOXP3, and IL-2Rβ [29, 30]. Likewise, the functional pathways for these signatures were determined to be vital to Treg development and activity between species. Receptors such as CCR2, CCR4, and CCR6, used for Treg trafficking to various tissues known in humans and rodents, are also enriched in canine Tregs [30]. Interactions of these receptors with CCL2 and CCL5 are responsible for orchestrating Treg migration to sites of tissue injury and inflammation or to secondary lymphoid organs, including lymph nodes. Once within these sites, Tregs can exert pleiotropic suppressive effects [39].

In the lymph nodes, Tregs inhibit the early stages of autoreactive T cells [40]. Likewise, Tregs recognize and downregulate autoreactive T cells and autoantigens by increasing the production of IL-10 and releasing immunomodulators such as IL-35 and TGF-β1 at the site of inflammation (**Figure 1**) [39]. For instance, TGF-β1 inhibits the effects of IL-17, a pro-inflammatory and neutrophil-mobilizing cytokine released by Th1 cells [41]. Additionally, IL-10 released by Tregs stimulates plasma cells to increase IgA production and inhibits IgM and IgG secretion (**Figure 1**) [42, 43]. For neuroprotection, Tregs and Th2 cells release IL-10, TGF-β1, IL-4, and IL-13 to transform M1 microglia into M2 microglia, thereby resisting inflammation within the CNS [44]. In contrast, M1 microglia support inflammation and elicit neurodegenerative effects through their production of reactive oxygen species (ROS), nitric oxide (NO), Fc receptors, and cytokines such as TNF-α, IL-1β, and IFNγ [44, 45]. These cytokines can cause further M1 microglia activation and induce reactive astrocytes (**Figure 1**) [44, 45]. When astrocytes are activated under inflammatory conditions, they can be involved in both neuroprotection and neurodegeneration; for instance, they can propagate inflammatory conditions by releasing IL-23, a cytokine that activates macrophages and maintains chronic autoimmune inflammation [44, 46]. IL-23 supports the release of IL-17, IL-22, and IFNγ by Th17 cells (**Figure 1**), thereby allowing these cells to release IL-21 and inhibit Tregs to create an autoimmune environment, neutrophil migration, and tumor growth [47]. In contrast, astrocytes and microglia can release CCL22 and CCL2 to attract CCR4-expressing cells, such as Th2 and Tregs, mitigating inflammation [36]. When a disease surpasses the limits and capabilities of Tregs, the microenvironment shifts to a pro-inflammatory state, risking further damage [8, 48].

Granulomatous meningoencephalomyelitis in dogs and multiple sclerosis

In dogs, noninfectious inflammatory diseases of the central nervous system (CNS) and/or meninges are known as meningoencephalitis of unknown origin (MUO) [49]. Canine patients are diagnosed with MUO when inflammation is present within the CNS and it does not have an infectious etiology [49]. MUO encompasses a variety of idiopathic inflammatory conditions of the canine CNS, including granulomatous

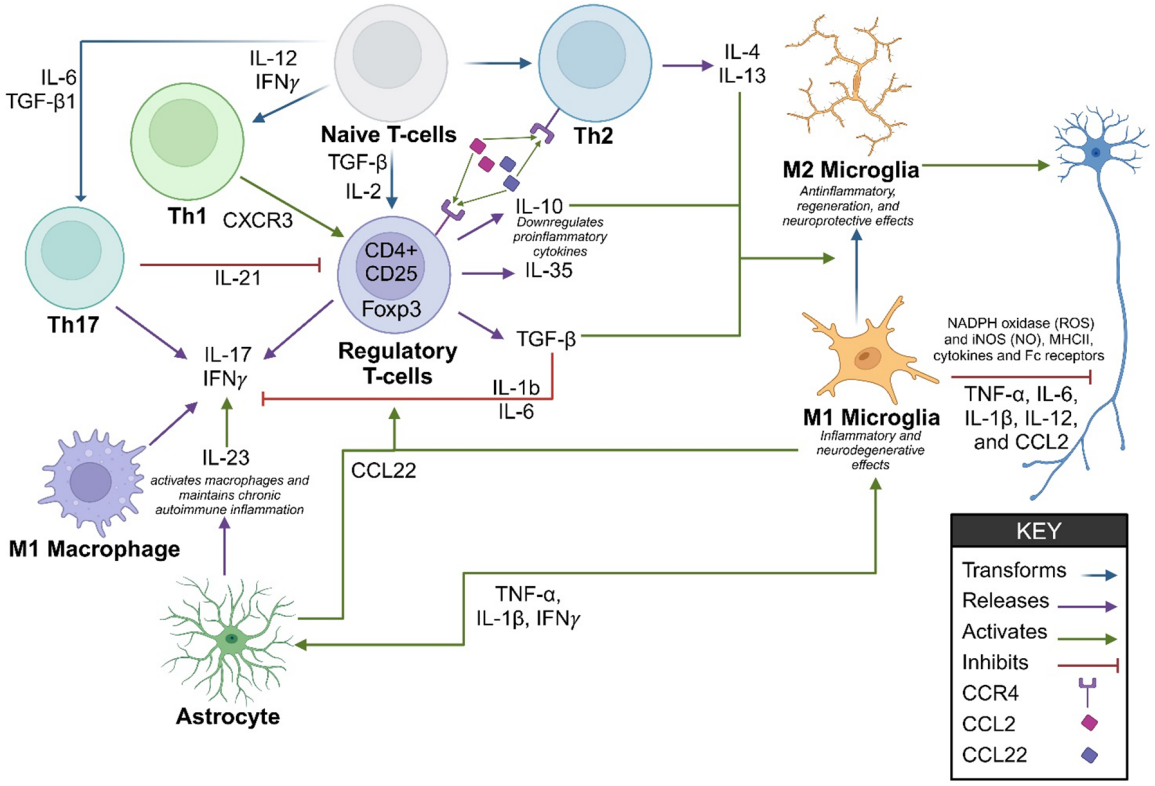

Figure 1. Regulatory activity of Tregs and their association with glial cells. Created with BioRender.com.

meningoencephalomyelitis (GME), necrotizing leukoencephalitis (NLE), and necrotizing meningoencephalitis (NME). A brain biopsy or post-mortem histopathology is required for a definite diagnosis of the MUO subtypes. However, the presumptive diagnosis of MUO can be accomplished antemortem by using clinical phenotype, magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis, and an infectious disease panel [49-51]. Nearly 36% of dogs presumptively diagnosed with MUO do not survive past 6 months since presentation, and about 36% of dogs did not have their clinical signs resolve despite medical intervention [52]. MUO subtypes have been investigated for Treg targets, which include effector T cells, their associated inflammatory mediators, and antibody production; this has revealed that the role of Treg in disease pathogenesis is largely unknown and, therefore, critically needed to assess therapeutic targets for MUO utilizing Treg functions.

GME was recently proposed as a natural model of MS due to histopathological similarities: B cell infiltration within the perivascular space, tertiary lymphoid organ-like structures forming within the leptomeninges, demyelination, and neuro-axonal injury [53, 54]. GME, similarly to MS, is an idiopathic, progressive neuroinflammatory disease that can present with mental changes, visual deficits, and spinal cord dysfunction in young female dogs [55-57]. In efforts to understand the pathogenesis of GME and identify clinically translatable biomarkers specific to the disease, previous studies have revealed elevated peripheral $CD4^+$ IL-17+ lymphocytes, increased IL-17 protein, and mRNA expression within brain tissue of GME patients (**Table 1**; **Figure 2**) [58, 59]. These results are highly indicative of Th17-mediated inflammation; however, IL-17 production is not limited to Th17 activity. $FoxP3^+$ Tregs have the capability to produce IL-17 when exposed to the pro-inflammatory cytokines IL-1β and IL-6 [60]. Though Tregs are known to regulate Th17 lymphocyte activity, Th17 cells can release IL-21, thereby suppressing Treg activity (**Table 1**) [61] which share a common precursor cell (the naive CD4 T cell) (**Table 1**) [61]. The mRNA expression of this cytokine is markedly elevated in dogs

Table 1. Shared features between multiple sclerosis in humans and granulomatous meningoencephalitis in dogs

Clinical	Molecular	Tregs
Prevalence among females. Idiopathic, progressive neuroinflammatory disease that can present with mentation changes, visual deficits, and spinal cord dysfunction.	B cell infiltration within the perivascular space. Tertiary lymphoid organ-like structures forming within the leptomeninges Demyelination and neuro-axonal injury.	Elevated $CD4^+$ $IL\text{-}17^+$ Lymphocytes Elevated IL-4 Elevated IL-17 Elevated IL-21

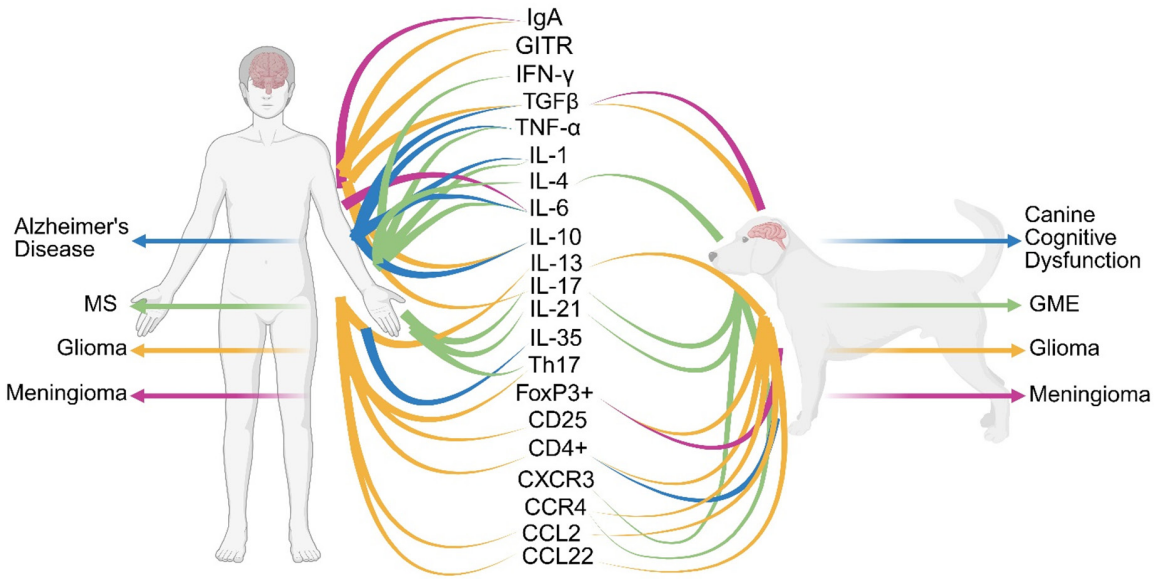

Figure 2. Inflammatory markers known to be associated with Treg activity and disease in both humans and dogs. The color of the lines and arrows corresponds to the pointed disease. Created with BioRender.com.

with GME and humans with MS, further supporting a pro-inflammatory pathogenesis [59].

Despite the pro-inflammatory environment of GME, immunosuppressive signatures have been identified and have been associated with Treg activity. Dogs diagnosed with GME had 70x greater CCR4 expression than dogs diagnosed with NME or NLE [59]. This receptor is found in both Th2 and Treg lymphocytes and promotes trafficking towards the target site by binding to its associated chemokine, CCL22 [36]. In MS, this chemokine was expressed at levels lower than healthy individuals, whereas experimental autoimmune encephalomyelitis (EAE), a mouse model for MS, had increased expression levels of both CCR4 and CL22 [36]. Treg chemotaxis is also mediated by CXCR3+; for instance, when CXCR3 was inhibited in mice with EAE, there was a decrease in Treg presence [62, 63]. This chemokine receptor is abundantly expressed in Th1 cells and certain macrophages/microglia. In dogs, CXCR3+ mRNA expression was markedly elevated in GME and NME compared to normal, healthy animals (**Figure 2**) [59]. The presence of CXCR3 may facilitate Tregs and other lymphocytes expressing this receptor to migrate to the site where CXCL9, CXCL10, and CXCL11 are being released [64]. Further supporting immunosuppressive efforts in GME, M1 microglia are promoted to transition to M2 microglia when exposed to IL-4, thereby mitigating further inflammation and eliciting neuroprotective effects [59]. Additionally, IL-4 is significantly higher in GME patients than in NME patients (**Figure 2**) [59, 65]. In human disease, IL-4 has been identified to be either starkly elevated or deficient in patients with MS and in EAE [66-69]. The variation of IL-4 in MS display the complexity of MS pathogenesis through IL-4 gene polymorphisms: the genotypes at the region of -590C/T differ between disease stages, such as in relapse-remitting MS (RRMS), and in dif-

ferent ethnic groups [70]. Both pro-inflammatory and anti-inflammatory markers are upregulated in dogs with GME compared to healthy controls. It is unknown to what degree each is elevated and how the dysfunction may result in the severe neuroinflammatory disease of GME or the different stages of MS.

Cognitive dysfunction in dogs and Alzheimer's disease

The prevalence of many neurodegenerative diseases has been steadily growing among both human and canine aging populations [71, 72]. Dementia affects nearly 55 million people worldwide, and Alzheimer's disease (AD) is the most common cause, affecting approximately two-thirds of patients with dementia, including nearly 7 million Americans [73, 74]. A proper understanding of disease progression and pathophysiology is required to develop therapeutic interventions, and the establishment of a complex disease model is critical [32]. Canine Cognitive Dysfunction (CCD) closely parallels AD, and is one of the most common causes of neural decline in older dogs [75]. AD and CCD are characterized by the presence of extracellular Aβ plaques in addition to neurofibrillary tangles in AD and phosphorylated tau protein in CCD [76, 77].

As in AD, the ability to remove Aβ plaques from the extracellular space within the neuroparenchyma diminishes as the dog ages, thereby resulting in pathologic accumulation [76]. Additionally, the plaques found in dogs share identical amino acid sequences with humans [77]. These plaques are deposited first within the prefrontal cortex in dogs, followed by the temporal, and then the occipital cortex [76]. In humans, early Aβ deposition occurs in the frontal cortex [76]. The location and degree of plaque deposition are significantly correlated with the onset and progression of clinical symptoms such as memory loss, disorientation, and interaction changes [75]. Progressive neuroaxonal loss and neurodegeneration, measured by the presence of free circulating neurofilament light-chain (NfL) in the plasma or cerebrospinal fluid (CSF) across both species, has also been reported [78, 79]. The described processes lead to the progression of neurodegeneration; however, recent evidence suggests that neuroinflammation might be the triggering factor [80]. As such, the translational value of CCD to AD offers a great opportunity to understand pathophysiology and develop treatment for both species.

In humans, early-onset microglia adopt an anti-inflammatory phenotype that can clear Aβ deposits [80-83]. As the disease progresses, microglia become more activated but have dysfunctional Aβ clearance abilities and elevated pro-inflammatory markers [80-83]. IL-10 has been shown to limit neuroinflammation by reducing damaging microglial activity [80, 84]. An additional regulator of neuroinflammation is TGF-β1. TGF-β1 prevents further Aβ production, tau-phosphorylation enzyme activity, and neuroinflammation, but it is decreased in the plasma of patients with AD [85, 86]. As the disease progresses, high levels of inflammatory markers surround Aβ plaques [80]. IL-1β has been shown to be upregulated throughout the disease, facilitating the production of TNF-α and IL-6 (**Figure 2**) [87]. Additionally, IL-1β has also been associated with the synthesis of amyloid precursor protein (APP) [88, 89]. The accumulation of APP and its synthesis into Aβ plaques propagates microglial activation and, therefore, IL-1β, TNF-α, and IL-6 production (**Figure 2**) [80, 90]. TNF-α upregulates vascular endothelial adhesion molecules, thereby facilitating leukocyte migration (**Figure 2**) [91]. Likewise, IL-6 is released by microglia and can inhibit further microglial activation or propagate microglial activation, leading to chronic neuroinflammation (**Figure 2**) [80, 92]. IL-6 has also been found to potentiate cyclin-dependent kinase 5 (CDK5) activity, resulting in the hyperphosphorylation of tau epitopes. Clinically, IL-6 is elevated in both CSF and serum in human patients with AD (**Figure 2**) [80, 93-95].

In dogs displaying clinical signs of cognitive dysfunction, these Aβ plaques were diffusely within deep cortical layers-as seen in end-stage AD-but had a mild presence of phagocytically active microglia throughout the tissue and lacked microglial aggregation around Aβ plaques [96, 97]. Instead, reactive astrocytes were predominantly associated with Aβ plaques [98, 99].

In murine models of AD, depleted and dysfunctional Tregs are associated with reduced microglial trafficking towards amyloid deposits, increased β plaque deposits, and acceleration

in cognitive deficits within APPPS1 and 3xTg-AD [100, 101]. Each of these is alleviated by FOXP3-expressing Treg activation and adoptive transfer of Tregs [100, 101]. Likewise, IL-2, an enhancer for Treg functional populations, is also associated with astrocytic and microglial activation around these amyloid plaques [102]. In humans with early-onset AD and mild cognitive impairment, Treg populations increase in association with IL-10, IL-35, and TGF-β elevations (**Figure 2**) [103-105]. As the disease progresses, peripheral $FOXP3^+$ Treg populations decrease [106]. The specific changes in Tregs in aging dogs are currently uncertain. Despite not knowing these trends in the canine population, the relative percentage of peripheral and naïve $CD4^+$ T cells decrease as a healthy dog ages (**Figure 2**) [107, 108].

Murine models of AD have demonstrated the neuroprotective roles of Tregs in mitigating cognitive decline [11, 106, 109]. The BBB becomes more permeable with age despite being known to limit the brain from overexposure to harmful materials [110]. As AD progresses, this permeability exacerbates, allowing more immune cells and chemokines to pass through into the neuroparenchyma [110]. P-glycoprotein (P-gp) is a transporter protein located within the endothelial cells that regulates toxins, microglia activation, and immune cell migration [110]. When assessed in humans with AD, there is an inverse association between Aβ accumulation and P-gp expression [110]. Normally, P-gp decreases with age, but AD and neuroinflammation exacerbate this rate of decrease [110, 111]. In dogs, there is a significant decline in P-gp within the BBB, placing the CNS at increased risk of harmful substances, neuroinflammation, and subsequent neurodegeneration [112].

The association of these natural aging patterns with immunologic responses has not been investigated in canines. Understanding the immunologic response within CCD guides in determining therapeutic potentials of Tregs and other cell subtypes; therefore there is great translational value between CCD and AD.

Canine and human glioblastoma

In humans, glioblastoma multiforme (GBM) is a highly aggressive primary brain tumor with patient survival that ranges from 12-24 months despite surgical management, radiation, and chemotherapy [113]. One of the major factors contributing to the aggressiveness of these tumors is the impairment of the host's anti-tumor immune response [113]. Glioblastoma cells can evade the immune system by releasing TGF-β and chemokines such as CCL2 and CCL22 to simultaneously suppress effector T cell responses, and enhance myeloid-derived suppressor cell (MDSCs) and microglia polarization as well as Treg migration towards the tumor site (**Figure 2**) [114]. In addition, TGF-β triggers Treg maturation by FOXP3 expression induction and acts as a signaling molecule in tumor invasion, migration, and immunosuppression (**Figure 2**) [9, 115, 116]. TGF-β's primary role lies in polarizing infiltrating glioma-associated microglia/macrophages (GAM) towards a tumor-supportive phenotype, thereby driving tumor progression [114, 117, 118]. Though the tumor microenvironment can become toxic to healthy brain resident cells, Tregs' differential gene expression allows them to survive in hypoxic environments [119]. Additionally, gliomas provide an environment conducive for Tregs to accumulate, proliferate, and suppress effector T cells [12]. To further assist the tumor evading immune recognition and destruction, Tregs inhibit IL-12 and INF-γ production from T cells infiltrating the tumor (**Figure 2**) [12]. Depletion of Tregs resulted in the restoration of effector T cell function in an orthotopic model of glioma, C57BL/6 mice orthotopically challenged with syngeneic GL261 cells [120]. Taken together, Tregs are heavily implicated in the immune evasion of GBM.

Canine high-grade gliomas (HGGs) develop *de novo* in an outbred, immunocompetent host and are increasingly being pursued as a naturally occurring model for the human GBM [121]. Canine HGGs, as defined by the Comparative Brain Tumor Consortium of the National Cancer Institute, encompass grade III and IV canine astrocytoma and oligodendroglioma [33]. With a comparable disease incidence, naturally occurring canine HGGs share clinical, imaging, and histopathologic features with human GBM [122]. Primarily manifesting in middle- to older-aged dogs, with a median diagnosis age of 8 years, gliomas display a mild predilection towards males and frequently localize within the olfactory bulb, temporal and parietal lobes,

with some invading into the ventricular system [123]. Brachycephalic breeds, such as the Boxer, Boston Terrier, and Bulldogs, are predisposed to HGG development [124, 125]. The mutations observed in both human and canine gliomas affect the same genes between the two species, and at nearly the same time points for the developing glioma: IDH1, PIK3CA, and EGFR at initiation or early event, respectively, and NF1 later in development [126]. Epigenetically, most of the studied canine gliomas had a methylation profile parallel to that of adult pediatrics despite these canine patients being beyond sexual maturity [126]. Tregs have been identified in high-grade canine oligodendroglioma and astrocytoma by immunohistochemistry [123]. They were dispersed in variable amounts throughout tumor tissues and sometimes extended past tissue borders, but were not present to this degree in healthy canine tissues [123]. In human studies, not only are $CD4^+FoxP3^+$ Tregs present within glioblastoma tumor tissues, but increased FOXP3 expression and decreased $CD4^+$ expression were associated with a greater the risk of disease recurrence; this demonstrates the complexity of Treg functionality [127] (**Figure 2**). Interestingly, TGF-β is upregulated at the junction of normal and neoplastic neuroparenchyma and associated with denser glioma associated macrophages and microglia (GAM) populations in both canine and human astrocytoma [128]. Additionally, glioma-derived CCL2 is a crucial chemoattractant responsible for Treg and GAM recruitment into the glioma microenvironment in murine and human tissues [121]. An increased CCL2 expression has been correlated with reduced overall survival in human glioma patients [129]. The antibody-mediated targeting of the Treg CCL2 high-affinity receptor, CCR4, reduced intertumoral Treg abundance and prolonged survival in an orthotopic murine glioma model [129, 130]. Recently, it has been demonstrated that CCL2 is robustly increased in canine HGG tumors relative to normal canine brains [121]. In addition, the CCL2-CCR4 signaling axis was proven to be necessary and sufficient for canine Treg chemotaxis towards canine glioma tissues (**Figure 1**) [121]. Blockade with CCR4 antagonist (C021) abrogated the migration of Tregs towards canine glioma supernatants [121]. The blockade of CCR4 with the FDA-approved monoclonal antibody reduced tumoral Treg infiltration and improved survival time in dogs affected with bladder and prostate cancer, and, therefore, exploring immunotherapeutic approaches targeting this axis in glioblastoma may hold promise for both canine and human patients [131, 132]. Alongside Tregs, additional inflammatory inhibitors found in humans and dogs with spontaneously occurring oligodendrogliomas include IL-13 receptor α2 (IL-13RA2) [133]. IL-13RA2 is a receptor that binds specifically to IL-13. This cytokine promotes anti-inflammatory phenotypes from microglia (**Figure 2**) [133]. Ongoing studies to dissect pathways governing Treg recruitment to the GBM microenvironment and how these cells contribute to immune suppression in GBM will identify more actionable therapeutic targets for GBM. Despite similarities between humans and dogs, our understanding of canine HGGs' intricate immune response pales in comparison to its human counterpart. While human gliomas have been extensively studied for their immune cell infiltration, including the recruitment of Tregs, this facet remains limited in canine gliomas [33]. Therefore, investigating Treg-mediated immune responses in dogs will add to the validation of the naturally occurring canine glioma model and to translation into human trials.

Canine and human meningioma

Meningiomas are the predominant primary brain tumor in dogs [134]. Alongside their histologic parallels with human meningiomas, canine meningiomas overexpress similar growth factor receptors, chromosomal deletions, and mutation events in tumor suppressor genes; for instance, mutations within PDGFRA, FOXM1, MYBL2, MK167, and/or other genes of the cell cycle pathway have been identified in both human and canine meningiomas [135-137]. This interspecies similarity thereby underscores spontaneously occurring canine meningiomas as a translational model for study [136, 137]. When assessing the presence and distribution of immune cells within canine meningiomas, not only were $FOXP3^+$ Tregs present in these tumors, but highly aggressive meningiomas contained greater densities of $FOXP3^+$ Tregs [134] (**Figure 2**). These dense populations of Tregs were not appreciated within non-diseased meninges [134]. Cytokines expressed within and around the tumor suggest an inflammatory response to the tumor [138].

The cytokine IL-6 promotes inflammatory responses by facilitating Th17 specification and other T effector cell activity [138]. In humans, IL-6 is increasingly produced within meningiomas *in vitro* (**Figure 2**) [138]. However, other studies display IL-6 acting as an inhibitor to tumor cell proliferation [139]. Immunohistochemistry on canine meningioma tissue displayed minimal IL-6 uptake [140]. Studies investigating changes in Treg homeostasis in dogs diagnosed with meningioma are largely lacking. Therefore, further investigation will greatly add to the validation of pet dogs as naturally occurring models for human studies.

Direct and indirect therapies targeting Tregs

With the ever-expanding knowledge of the integral role Tregs play in various neurologic diseases, clinical studies have advanced into applying Treg-related interleukins and antibodies to either enhance or deplete immune responses. The diseases requiring increasing populations and activity of Tregs include MS and AD [141]. Teriflunomide, a pyrimidine synthase inhibitor and active metabolite of leflunomide, is a drug that is approved for relapsing and remitting multiple sclerosis and active secondary progressive multiple sclerosis in adult patients [21]. From the results of stage IV clinical trials regarding the effect on Tregs, CD39 was increased within Tregs, and CXCR3 was decreased in T helper cells after treatment with teriflunomide (see **Table 2**, NCT03464448). CD39 is known to be associated with enhancing Tregs' immunosuppressive function, while CXCR3 has been positively correlated with inflammatory responses [22]. These findings, alongside other immunomodulatory effects, identified an increase in tolerogenic effects in contrast to a pro-inflammatory environment [22]. In directly targeting B and T lymphocytes, alemtuzumab has been approved for relapsing-remitting multiple sclerosis for its target against CD52 and restoring an immunotolerant environment [23]. The phase IV study is exploring alemtuzumab's immune signatures and thereby the mechanism of action within peripheral blood and CSF (see **Table 2**, NCT02419378). Its effects on immune regulation revealed increasing populations of $CD4^{+}CD25^{high}CD127^{low}$ Tregs alongside increased levels of TGFβ-1 and IL-10, immunoregulatory cytokines, and reduction of IFN-γ, IL-12, IL-17, IL-21, IL-23, and IL-27, pro-inflammatory cytokines within serum [23-26]. To target the migration of activated lymphocytes, natalizumab has been proposed for the treatment of MS. Natalizumab is a monoclonal antibody against integrin α4β1 and has advanced into stage IV clinical trials for MS (see **Table 2**, NCT00859482) [27]. This antibody targets VLA-4 at the endothelium of the blood-brain barrier in order to suppress lymphocytic transmigration [27]. In a preliminary study analyzing the lymphocytic populations of individuals diagnosed with RRMS, natalizumab did not affect the proportion of $CD4^{+}CD25^{++}FoxP3^{+}$ Treg within the peripheral blood after 12 months of treatment [27]. However, this study acknowledged severe rebound effects of elevated lymphocytic migration after discontinuing the drug [27]. Modifying Tregs themselves has begun its early stages of clinical trials in humans with progressive MS. ABA-101 is an autologous Treg therapy that is designed to target damaged myelin induced by progressive MS and exert immunoregulatory effects within the region (see **Table 2**, NCT06566261) [28]. This therapy has recently been granted fast-track status by the United States Food and Drug Administration [28]. Directly modifying a significant component of MS progression can lead to a further understanding of the significance of Tregs in disease pathogenesis, progression, and mitigation, including adaptation for other inflammatory neurologic diseases. The approach to AD using Tregs has similar goals to MS: support immune regulation to prevent the progression of inflammatory disease. Currently in phase II clinical trials, IL-2 has been adopted as a disease-modifying biologic to restore Treg function, thereby facilitating a tolerogenic environment (see **Table 2**, NCT06096090, NCT05468073). This cytokine is also in phase II clinical trials for use in patients with MS [142].

On the contrary, Tregs have been appreciated in significant numbers within and around glioma and meningiomas and are suspected of sustaining tumor growth. While IL-2 has been a target to increase Treg populations and functions for immunosuppressive purposes, IL-2 has also been targeted for immune-enhancing purposes. Basiliximab, a monoclonal antibody currently in phase I clinical trials (see **Table 2**, NCT00626483, NCT00626015), binds to the IL-2 receptor alpha chain (CD25), thereby tar-

Table 2. Current therapies and interventions undergoing clinical trials assessing the effect on Tregs for different neurologic diseases as of January 2025

Clinical Trials Aimed at Tregs and Treg-Associated Pathways						
ClinicalTrials.gov identifier Trial Title	Disease	Phase	Intervention	Drug Class	Target	Effect on T cells
NCT06671236 Clinical Study of Regulatory T Cells (Tregs) in the Treatment of Neurodegenerative Diseases	Multiple Sclerosis	I	Biological: Autologous Human Polyclonal Regulatory T Cells Injection (NP001 Cell Injection)	Biologic drug	Immunomodulation	Prevents aberrant immune activation associated with autoimmunity.
NCT06566261 ABA-101 in Participants with Progressive Multiple Sclerosis	Multiple Sclerosis	I	Biological: ABA-101	Biologic drug	Damaged myelin	ABA-101 is a T-cell receptor engineered, autologous Treg therapy
NCT02035514 Phase I-II Clinical Trial With Autologous Bone Marrow Derived Mesenchymal Stem Cells for the Therapy of Multiple Sclerosis	Multiple Sclerosis	I-II	Biological: Bone marrow autologous mesenchymal stem cells transplantation	Biologic drug	Peripheral immunomodulation and stimulate lesion repair and regeneration	Inhibits T cell-mediated cytolysis, proliferation, and regulates IL-1 and IFN-γ for Th1/Th2 balance; promotes proliferation of CD4(+) CD25(high) FOXP3(+) regulatory T cells.
NCT02166021 Clinical Efficacy of Autologous Mesenchymal Bone Marrow Stem Cells in Active & Progressive Multiple Sclerosis	Multiple Sclerosis	II	Biological: Mesenchymal stem cells	Biologic drug	Immunomodulation	Prevents autoimmune phenotypes of $CD4^+$ and $CD8^+$ T cell activation; induces Treg maturation and supports anti-inflammatory activity.
NCT02424396 Biological Activity and Safety of Low Dose IL2 in Relapsing Remitting Multiple Sclerosis	Multiple Sclerosis	II	Drug: IL-2	Biologic Response Modifier	Immunomodulation: IL-2RG	Promotes T-cell proliferation and differentiation of regulatory T cells.
NCT04530318 Dendritic Cells Therapy Combined With Immunomodulatory Treatment in Multiple Sclerosis	Multiple Sclerosis	II	Other: Autologous peripheral blood differentiated adult tolerogenic dendritic cells expanded	Biologic drug	Immunomodulation	Supports Treg maturation and activity.
NCT01665144 Exploring the Efficacy and Safety of Siponimod in Patients With Secondary Progressive Multiple Sclerosis	Multiple Sclerosis	III	Drug: Siponimod (BAF312)	N-alylindoles: Selective sphingosine 1-phosphate receptor modulator	Immunomodulation: G-protein-coupled receptors (GPCRs)	Inhibit Th17 differentiation and increase Treg differentiation.
NCT02419378 Alemtuzumab in Autoimmune Inflammatory Neurodegeneration: Mechanisms of Action and Neuroprotective Potential	Multiple Sclerosis	IV	Drug: Alemtuzumab	Monoclonal antibody	Immunomodulation: CD52-expressing B and T cells	Depletes CD-52-expressing T cells and supports differentiation of regulatory T cells.
NCT00859482 Differential Immune Effects of Natalizumab	Multiple Sclerosis	IV	Drug: Natalizumab	IgG4κ monoclonal antibody	Immunomodulation: α4-integrin	Reduce leukocyte migration into and surveillance within the CNS.
NCT03464448 Mechanistic Studies of Teriflunomide in RRMS	Multiple Sclerosis	IV	Drug: Teriflunomide	Pyrimidine synthesis inhibitor	Anti-inflammatory and immunomodulation	Reduce $CD8^+$ T cell proliferation, proinflammatory function, and migration.
NCT05821153 Low Dose IL2 Immunotherapy in AD	Alzheimer's Disease	I	Drug: Aldesleukin	Biologic Response Modifier	Immunomodulation: IL-2RG	Promotes T-cell proliferation and differentiation of regulatory T cells.
NCT06671236 Clinical Study of Regulatory T Cells (Tregs) in the Treatment of Neurodegenerative Diseases	Alzheimer's Disease	I	Biological: Autologous Human Polyclonal Regulatory T Cells Injection (NP001 Cell Injection)	Biologic drug	Immunomodulation	Prevents aberrant immune activation associated with neurodegeneration.

NCT06096090 Phase II Clinical Trial of Interleukin-2 in AD	Alzheimer's Disease	II	Drug: Interleukin-2	Biologic Response Modifier	Immunomodulation: IL-2RG	Promotes T-cell proliferation and differentiation of regulatory T cells.
NCT05468073 Therapeutic Evaluation of Low-dose IL-2-based Immunomodulatory Approach in Patients With Early AD	Alzheimer's Disease	II	Drug: Proleukin (Aldesleukin)	Biologic Response Modifier	Immunomodulation: IL-2RG	Promotes T-cell proliferation and differentiation of regulatory T cells.
NCT02718443 VXM01 Phase I Pilot Study in Patients With Operable Recurrence of a Glioblastoma	Glioma	I	Drug: VXM01	DNA T-Cell Vaccine	Vascular endothelial growth factor (VEGF) receptor 2	Activates $CD4^+$ T helper cells against VEGF Receptor 2.
NCT01920191 Phase I/II Trial of IMA950 Multi-peptide Vaccine Plus Poly-ICLC in Glioblastoma	Glioma	I	Biological: IMA 950 (9 MHC class I and 2 MHC class II peptides)	Tumor-associated peptide vaccine	T cells	Production of $CD8^+$ T-cell immune responses specific against gliomas (brevican (BCAN), chondroitin sulfate proteoglycan 4 (CSPG4), fatty acid binding protein 7 (FABP7), insulin like growth factor 2 mRNA binding protein 3 (IGF2BP3), neuronal cell adhesion molecule (NRCAM), neuroligin 4 X-linked (NLGN4X), protein tyrosine phosphatase, receptor, 2 HLA DR- binding peptides type Z1 (PTPRZ1), and tenascin C (TNC)).
NCT00626015 Chemotherapy, Radiation Therapy, and Vaccine Therapy With Basiliximab in Treating Patients With Glioblastoma Multiforme That Has Been Removed by Surgery	Glioma	I	Drug: Basiliximab	Monoclonal antibody	CD25 on activated effector and regulatory T cells	Inhibits and depletes Treg populations and sensitizes Treg to IL-7.
NCT00626483 Basiliximab in Treating Patients With Newly Diagnosed Glioblastoma Multiforme Undergoing Targeted Immunotherapy and Temozolomide-Caused Lymphopenia	Glioma	I	Drug: Basiliximab	Monoclonal antibody	CD25 on activated effector and regulatory T cells	Inhibits and depletes Treg populations and sensitizes Treg to IL-7.
NCT01091792 Exploratory Study of the Modulation of the Immune System by VEGF Blockade in Patients With Glioblastoma Multiforme (GBM)	Glioma	I	Drug: Bevacizumab	Monoclonal antibody	Vascular endothelial growth factor (VEGF)	Actives and enhances CD8 T cell tumor infiltration and decreases immunosuppressive Tregs.
NCT02669173 Capecitabine + Bevacizumab in Patients With Recurrent Glioblastoma	Glioma	I	Drug: Bevacizumab + Capecitabine	Monoclonal antibody	Vascular endothelial growth factor (VEGF)	Actives and enhances CD8 T cell tumor infiltration and decreases immunosuppressive Tregs.
NCT04642937 Study of CD200 Activation Receptor Ligand (CD200AR-L) and Allogeneic Tumor Lysate Vaccine Immunotherapy for Recurrent Glioblastoma	Glioma	I	Drug: hP1A8	Activation receptor ligand adjuvant	CD200	Activates antigen-presenting cells, release pro-inflammatory cytokines, and fails to induce Treg activation.
NCT03688178 DC Migration Study to Evaluate TReg Depletion In GBM Patients With and Without Varlilumab	Glioblastoma	II	Drug: Varlilumab	Monoclonal antibody	CD27	Enhances CD-27 T cell pathway, induces $CD8^+$ T cell reactivity, and depletes Treg populations.
NCT00323115 Phase II Feasibility Study of Dendritic Cell Vaccination for Newly Diagnosed Glioblastoma Multiforme	Glioma	II	Autologous Dendritic Cell	Biologic drug	Glioma antigens	Enhances antigen-presenting cell interaction with T cells, augments CD+ T memory cells.

NCT01920191 Phase I/II Trial of IMA950 Multi-peptide Vaccine Plus Poly-ICLC in Glioblastoma	Glioma	II	Biological: Polyinosinic acid-polycytidylic acid stabilized with polylysine (poly-ICLC)	Synthetic dsRNA analogue	Toll-like receptor 3, RIG-I-like receptor (MDA5), and other pattern recognition receptors (PRRs) signaling	Activates $CD8^+$ T cells, promotes effector T cell infiltration, upregulates chemokine transcription and antigen presentation, and induces type I and II interferons.
NCT01836536 Search for a Link Between Response to Treatment and Circulating Leucocytes in High Grade Glioma Patients	Glioma	II	Drug: Bevacizumab	Monoclonal antibody	Vascular endothelial growth factor (VEGF)	Actives and enhances CD8 T cell tumor infiltration and decreases immunosuppressive Tregs.
NCT01903330 ERC1671/GM-CSF/Cyclophosphamide for the Treatment of Glioblastoma Multiforme	Glioma	II	Drug: (ERC1671/GM-CSF/Cyclophosphamide)+bevacizumab/bevacizumab biosimilar	Sargramostim-alkylating agent-antineoplastic + Monoclonal Antibody.	Cyclophosphamide: immunosuppressant; Bevacizmab: Monoclonal antibody	Cyclophosphamide: Depletes Treg populations; Bevacizumab: Actives and enhances CD8 T cell tumor infiltration and decreases immunosuppressive Tregs.
NCT02648997 An Open-Label Phase II Study of Nivolumab or Nivolumab/Ipilimumab in Adult Participants With Progressive/Recurrent Meningioma	Meningioma	II	Drug: Nivolumab	IgG4 antibody	Tumor programmed death ligand 1 (PD-L1)	Inhibits PD-L1-induced $CD4^+CD25^+FOXP3^+$ Treg proliferation.
NCT02035514 Phase I-II Clinical Trial With Autologous Bone Marrow Derived Mesenchymal Stem Cells for the Therapy of Multiple Sclerosis	Multiple Sclerosis	I-II	Biological: Bone marrow autologous mesenchymal stem cells transplantation	Biologic drug	Peripheral immunomodulation and stimulate lesion repair and regeneration	Inhibits T cell-mediated cytolysis, proliferation, and regulates IL-1 and IFN-γ for Th1/Th2 balance; Treg: promotes proliferation of CD4(+) CD25(high) FOXP3(+) regulatory T cells.

geting Tregs [16]. Since Tregs largely rely on CD25 for their immunoregulatory functions and proliferation, basiliximab impairs Treg function [16]. Tregs have become a marker for treatment efficacy and prognosis in current clinical trials. An example is seen with long-term treatment with temozolomide, an alkylating agent, for glioblastoma, which can increase the risk of individuals undergoing a "rebound phenomenon" where Treg populations acutely rise [17]. A common drug currently being evaluated for the treatment of human gliomas is bevacizumab (see **Table 2**, NCT02669173, NCT01091792), a monoclonal antibody that targets vascular endothelial growth factors (VEGF) that are highly expressed in glioma [15]. However, there have been inaccurate representations of circulating VEGF for prognosis after treatment. Instead, populations of circulating $CD3^+CD4^+CD25^+FOXP3^+$ Tregs were strongly correlated with prognosis after treatment with bevacizumab: treatment resulted in significantly lower $Foxp3^+$ Tregs than in those without treatment [15]. Further enhancement of the the efficacy of bevacizumab can be achieved by targeting Tregs using anti-$CD25^+$ antibodies [18]. Using this combined therapy, murine models with glioma significantly improved survival, possibly restoring $CD8^+$ T cell function [18]. For meningioma, nivolumab, an IgG4 antibody, is being investigated within stage II clinical trials as a target against tumor-programmed death ligand 1 (PD-L1) within meningiomas (see **Table 2**, NCT02648997). The microenvironment of these tumors has increased expression of PD-L1 in addition to the immunosuppressive activated Tregs [19, 20]. Nivolumab has been associated with preventing Treg proliferation and increasing the rate of progression-free survival as a monotherapy and as a combination therapy [19, 20].

Canine model successes

Canine models are a new, but effective preclinical model for human clinical trials, showing a number of successes. Novel therapeutics can be adopted, and their involvement in the animal's physiologic responses can be evaluated based on their genetic heterogeneity, similar biomarkers, and histologic features of disease, complex immune responses, and similar environmental exposures. Dogs undergoing novel therapies may undergo full systemic evaluation for on-target and off-target effects to be used as predictors of human responses during clinical trials. This full systemic evaluation includes assessing immunologic responses to both therapy and the disease that may otherwise be limited in immunocompromised murine models.

More than 30% of human and canine primary osteosarcomas (OSA) contain membrane-bound receptor tyrosine kinase (HER2) and are associated with aggressive forms of OSA [143]. *Listeria monocytogenes* (*Lm*) was engineered to express HER2 epitopes fused with lysin listeriolysin O (ADXS31-164) to induce anti-tumor T cell responses. By administering ADXS31-164, peripheral tolerance of HER2-concentrating tissues is compromised, leading to T-cell-mediated tissue regression [143]. Tissue regression decreased metastasis, and overall improved survival was found in rodent models with OSA after administering ADXS31-164. This novel therapy underwent additional investigations to determine remission longevity and characteristics of the immune response against ADXS31-164. For this more comprehensive approach, investigators delivered ADXS31-164 to the species most synonymous with human OSA, dogs [143]. Treated dogs displayed clinical signs associated with ADXS31-164 near the time of administration, primarily consisting of gastrointestinal indiscretion (e.g. nausea, anorexia, lethargy) [143]. Additionally, dogs and humans treated with ADXS31-164 experienced similar challenges that may be addressed for improvements such as immunity against CAR T therapy and poor CAR T proliferation [143]. Interestingly, survival rate was determined based on the immunologic profile of short-term survivors versus long-term survivors. These long-term survivors experience an increase in body temperature and increase in serum cytokines (IL-6, TNF-α, IFN-γ and MCP-1), supporting the necessity of immunologically immune models [143]. These findings facilitated the progress of ADXS31-164 (OST31-164) into clinical trials which are currently in the follow up phase of phase IIb (NCT04974008).

Humans and dogs share a grave prognosis of highly aggressive gliomas. The immunosuppressive environment to support glioblastomas involves the immune checkpoint protein for T-cell activation, CD200 [144, 145]. When CD200 is released by the tumor, CD200 inhib-

its the immune response by binding to the inhibitory receptor on antigen-presenting cells, inhibits the release of pro-inflammatory cytokines, and activates Tregs [144, 145]. In contrast, there are activation receptor ligands (CD200AR) for CD200. When CD200AR is bound to CD200, immature dendritic cells begin to differentiate and produce cytokines aimed at T-cell mediated response [144]. Peptides of CD200 were developed as ligands for CD200AR (CD200AR-L) to reverse the immunosuppressive environment of gliomas [144, 145]. To achieve this, the CD200AR-L was combined with autologous tumor lysate vaccines and administered subcutaneously in dogs after glioma resection [144]. The median survival time of dogs with this combined treatment was 12.9 months, nearly double the survival of 6.8 months in dogs who only received the tumor lysate vaccine [144, 145]. Alongside the increased survival, dogs receiving this combined treatment lacked adverse clinical signs; thereby supporting its progression into Phase I clinical trials (see **Table 2**, NCT04642937) [145].

Limitations of canine models

The cultural and practical differences between veterinary and human medicine present challenges to advancing veterinary clinical studies. The socio-demographics of institutions conducting clinical trials can influence the medical decisions and follow-up care for animals with a given disease [146]. Owners with limited financial resources may opt for euthanasia instead of pursuing costly diagnostic procedures or extensive care, leading to underreporting of certain diseases [146]. In cases where owners decide to pursue further diagnostic workup and treatment, the financial and/or medical burden can be significant; for instance, veterinary patients' comorbidities, such as severe cardiovascular disease may deter owners and clinicians from performing fully sedated diagnostic procedures, particularly if the suspected condition is not life-threatening to the degree of imminent cardiac arrest. The frequency of follow-up visits can be influenced by the animal's behavior and the owner's availability [146]. Transporting the animal before, during, and after a veterinary visit can place a physical, emotional, and financial burden on an owner; for instance, the owner may require taking time off work to transport their large-breed dog with an orthopedic condition to the veterinary hospital [146]. The animal's behavior while at the veterinary hospital may place additional limitations on its length of stay and the frequency of performing certain diagnostics and degree of assessment; this may require the addition of anxiolytics and/or sedatives [146]. These factors can contribute to loss of follow-up. Research groups with greater funding do better with incentivizing participation and follow-up, but this can place additional strain on smaller, less funded research groups [146].

When designing the study, additional factors to consider in veterinary medicine include breed-specific differences in each species, castration status, and age at the time of castration. Certain medical conditions are known to be highly correlated to the length of certain hormonal exposure and/or certain breeds of our canine patients; for instance, sexually intact dogs, particularly smaller dogs, were associated with lower odds of orthopedic disease and lower odds of obesity [147, 148]. Events that may have preceded the animal's adoption or rescue are at greater risk of being lost in the veterinary population; for instance, the age at castration, health condition of the littermates, and even breed [149].

Conclusion

Tregs are important regulators of immune homeostasis that play a role in the development and progression of autoimmune disease, cancer, and tissue repair [8-10, 150]. Treg-targeted therapies are under development and clinical investigation as a standalone or adjunctive therapeutic modality are ongoing [15-28, 142]. While the rationale for targeting Tregs in these diseases is compelling, the clinical results have often not fulfilled the promise of such an approach to date. Current models often lack the natural occurrence of these diseases and/or are immune-compromised. This dissimilarity can influence the understanding of the pathophysiologic onset and progression of these neurologic diseases. Gaps in this understanding can thereby risk clinical trial failure. It is imperative to identify and bolster current pre-clinical models by investigating changes in Treg homeostasis in naturally occurring and immune-competent hosts such as pet dogs.

Detailed analysis of longitudinal changes in Treg homeostasis in dogs with CNS disease of high translational value to humans will allow meaningful research leading to successful trials with mutual benefit to both humans and dogs.

Disclosure of conflict of interest

None.

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References

[1] Feigin VL, Vos T, Nichols E, Owolabi MO, Carroll WM, Dichgans M, Deuschl G, Parmar P, Brainin M and Murray C. The global burden of neurological disorders: translating evidence into policy. Lancet Neurol 2019; 19: 255-265.

[2] Grech N, Dalli T, Mizzi S, Meilak L, Calleja N and Zrinzo A. Rising incidence of glioblastoma multiforme in a well-defined population. Cureus 2020; 12: e8195.

[3] Walton C, King R, Rechtman L, Kaye W, Leray E, Marrie RA, Robertson N, Rocca NL, Uitdehaag B, Mei I van der, Wallin M, Helme A, Napier CA, Rijke N and Baneke P. Rising prevalence of multiple sclerosis worldwide: insights from the Atlas of MS, third edition. Mult Scler 2020; 26: 1816-1821.

[4] Tizard I. Chapter 7 - Autoimmune Neurologic Diseases. In: Tizard I, editor. Autoimmune Diseases in Domestic Animals. New Delhi: Elsevier; 2023. p. 77-94.

[5] Norris GT and Kipnis J. Immune cells and CNS physiology: microglia and beyond. J Exp Med 2019; 216: 60-70.

[6] Wang CX and Shuaib A. Involvement of inflammatory cytokines in central nervous system injury. Prog Neurobiol 2002; 67: 161-172.

[7] Kerschensteiner M, Meinl E and Hohlfeld R. Neuro-immune crosstalk in CNS diseases. Neuroscience 2009; 158: 1122-1132.

[8] Duffy SS, Keating BA, Perera CJ and Moalem-Taylor G. The role of regulatory T cells in nervous system pathologies. J Neurosci Res 2018; 96: 951-968.

[9] Sakaguchi S, Yamaguchi T, Nomura T and Ono M. Regulatory T cells and immune tolerance. Cell 2008; 133: 775-787.

[10] Kondělková K, Vokurková D, Krejsek J, Borská L, Fiala Z and Andrýs C. Regulatory T cells (Treg) and their roles in immune system with respect to immunopathological disorders. Acta Medica (Hradec Kralove) 2010; 53: 73-77.

[11] Olson KE, Mosley RL and Gendelman HE. The potential for treg-enhancing therapies in nervous system pathologies. Clin Exp Immunol 2023; 211: 108-121.

[12] Humphries W, Wei J, Sampson JH and Heimberger AB. The role of tregs in glioma-mediated immunosuppression: potential target for intervention. Neurosurg Clin N Am 2010; 21: 125-137.

[13] Jacobs JF, Idema AJ, Bol KF, Grotenhuis JA, de Vries IJ, Wesseling P and Adema GJ. Prognostic significance and mechanism of Treg infiltration in human brain tumors. J Neuroimmunol 2010; 225: 195-199.

[14] Heimberger AB, Kong LY, Abou-Ghazal M, Reina-Ortiz C, Yang DS, Wei J, Qiao W, Schmittling RJ, Archer GE, Sampson JH, Hiraoka N, Priebe W, Fuller GN and Sawaya R. The role of tregs in human glioma patients and their inhibition with a novel STAT-3 inhibitor. Clin Neurosurg 2009; 56: 98-106.

[15] Quillien V, Carpentier AF, Gey A, Avril T, Tartour E, Sejalon F, Campillo-Gimenez B and Vauleon E. Absolute numbers of regulatory T cells and neutrophils in corticosteroid-free patients are predictive for response to bevacizumab in recurrent glioblastoma patients. Cancer Immunol Immunother 2019; 68: 871-882.

[16] López-Abente J, Martínez-Bonet M, Bernaldo-de-Quirós E, Camino M, Gil N, Panadero E, Gil-Jaurena JM, Clemente M, Urschel S, West L, Pion M and Correa-Rocha R. Basiliximab impairs regulatory T cell (TREG) function and could affect the short-term graft acceptance in children with heart transplantation. Sci Rep 2021; 11: 827.

[17] Tamura R, Tanaka T, Ohara K, Miyake K, Morimoto Y, Yamamoto Y, Kanai R, Akasaki Y, Murayama Y, Tamiya T, Yoshida K and Sasaki H. Persistent restoration to the immunosupportive tumor microenvironment in glioblastoma by bevacizumab. Cancer Sci 2019; 110: 499-508.

[18] Long Y, Tao H, Karachi A, Grippin AJ, Jin L, Chang YE, Zhang W, Dyson KA, Hou AY, Na M, Deleyrolle LP, Sayour EJ, Rahman M, Mitchell DA, Lin Z and Huang J. Dysregulation of glutamate transport enhances treg function that promotes VEGF blockade resistance in glioblastoma. Cancer Res 2020; 80: 499-509.

[19] Bi WL, Nayak L, Meredith DM, Driver J, Du Z, Hoffman S, Li Y, Lee EQ, Beroukhim R, Rinne M, McFaline-Figueroa R, Chukwueke U, McCluskey C, Gaffey S, Cherniack AD, Stefanik J, Doherty L, Taubert C, Cifrino M, LaFrankie D, Graillon T, Wen PY, Ligon KL, Al-Mefty O, Huang

RY, Muzikansky A, Chiocca EA, Santagata S, Dunn IF and Reardon DA. Activity of PD-1 blockade with nivolumab among patients with recurrent atypical/anaplastic meningioma: phase II trial results. Neuro Oncol 2022; 24: 101-113.
[20] DiDomenico J, Lamano JB, Oyon D, Li Y, Veliceasa D, Kaur G, Ampie L, Choy W, Lamano JB and Bloch O. The immune checkpoint protein PD-L1 induces and maintains regulatory T cells in glioblastoma. Oncoimmunology 2018; 7: e1448329.
[21] Aly L, Hemmer B and Korn T. From leflunomide to teriflunomide: drug development and immunosuppressive oral drugs in the treatment of multiple sclerosis. Curr Neuropharmacol 2017; 15: 874-891.
[22] Wu Q, Wang Q, Yang J, Mills EA, Chilukuri P, Saad A, Dowling CA, Fisher C, Kirch B and Mao-Draayer Y. Teriflunomide modulates both innate and adaptive immune capacities in multiple sclerosis. Mult Scler Relat Disord 2023; 75: 104719.
[23] Rolla S, Maglione A, De Mercanti SF and Clerico M. The meaning of immune reconstitution after alemtuzumab therapy in multiple sclerosis. Cells 2020; 9: 1396.
[24] Freedman MS, Kaplan JM and Markovic-Plese S. Insights into the mechanisms of the therapeutic efficacy of alemtuzumab in multiple sclerosis. J Clin Cell Immunol 2013; 4: 1000152.
[25] Cox AL, Thompson SA, Jones JL, Robertson VH, Hale G, Waldmann H, Compston DA and Coles AJ. Lymphocyte homeostasis following therapeutic lymphocyte depletion in multiple sclerosis. Eur J Immunol 2005; 35: 3332-3342.
[26] Jones JL, Thompson SA, Loh P, Davies JL, Tuohy OC, Curry AJ, Azzopardi L, Hill-Cawthorne G, Fahey MT, Compston A and Coles AJ. Human autoimmunity after lymphocyte depletion is caused by homeostatic T-cell proliferation. Proc Natl Acad Sci U S A 2013; 110: 20200-20205.
[27] Tanasescu R, Frakich N, Chou IJ, Filippini P, Podda G, Xin G, Muraleedharan R, Jerca O, Onion D and Constantinescu CS. Natalizumab treatment of relapsing remitting multiple sclerosis has no long-term effects on the proportion of circulating regulatory T cells. Neurol Ther 2023; 12: 2041-2052.
[28] Goischke HK. Will there be enough evidence to offer Vitamin D supplementation to patients with multiple sclerosis in 2024? J Neurol Res Rev Rep 2024; 6: 1-22.
[29] Garden OA, Pinheiro D and Cunningham F. All creatures great and small: regulatory T cells in mice, humans, dogs and other domestic animal species. Int Immunopharmacol 2011; 11: 576-588.
[30] Wu Y, Chang YM, Stell AJ, Priestnall SL, Sharma E, Goulart MR, Gribben J, Xia D and Garden OA. Phenotypic characterisation of regulatory T cells in dogs reveals signature transcripts conserved in humans and mice. Sci Rep 2019; 9: 13478.
[31] Constantinescu CS, Farooqi N, O'Brien K and Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol 2011; 164: 1079-1106.
[32] Chen ZY and Zhang Y. Animal models of Alzheimer's disease: applications, evaluation, and perspectives. Zool Res 2022; 43: 1026-1040.
[33] Koehler JW, Miller AD, Miller CR, Porter B, Aldape K, Beck J, Brat D, Cornax I, Corps K, Frank C, Giannini C, Horbinski C, Huse JT, O'Sullivan MG, Rissi DR, Mark Simpson R, Woolard K, Shih JH, Mazcko C, Gilbert MR and LeBlanc AK. A revised diagnostic classification of canine glioma: towards validation of the canine glioma patient as a naturally occurring preclinical model for human glioma. J Neuropathol Exp Neurol 2018; 77: 1039-1054.
[34] Hegedus C, Andronie L, Uiuiu P, Jurco E, Lazar EA and Popescu S. Pets, genuine tools of environmental pollutant detection. Animals (Basel) 2023; 13: 2923.
[35] Zhang H, Kong H, Zeng X, Guo L, Sun X and He S. Subsets of regulatory T cells and their roles in allergy. J Transl Med 2014; 12: 125.
[36] Cui LY, Chu SF and Chen NH. The role of chemokines and chemokine receptors in multiple sclerosis. Int Immunopharmacol 2020; 83: 106314.
[37] McHugh RS, Shevach EM and Thornton AM. Control of organ-specific autoimmunity by immunoregulatory CD4(+)CD25(+) T cells. Microbes Infect 2001; 3: 919-927.
[38] Brisslert M, Bokarewa M, Larsson P, Wing K, Collins LV and Tarkowski A. Phenotypic and functional characterization of human CD25+ B cells. Immunology 2006; 117: 548-57.
[39] Campbell DJ. Control of regulatory T cell migration, function, and homeostasis. J Immunol 2015; 195: 2507-13.
[40] Kawahata K, Misaki Y, Yamauchi M, Tsunekawa S, Setoguchi K, Miyazaki J and Yamamoto K. Generation of CD4(+)CD25(+) regulatory T cells from autoreactive T cells simultaneously with their negative selection in the thymus and from nonautoreactive T cells by endogenous TCR expression. J Immunol 2002; 168: 4399-4405.
[41] Afzali B, Lombardi G, Lechler RI and Lord GM. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol 2007; 148: 32-46.

[42] Meiler F, Klunker S, Zimmermann M, Akdis CA and Akdis M. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. Allergy 2008; 63: 1455-1463.
[43] Taylor A, Verhagen J, Blaser K, Akdis M and Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-β: the role of T regulatory cells. Immunology 2006; 117: 433-42.
[44] Guo S, Wang H and Yin Y. Microglia polarization from M1 to M2 in neurodegenerative diseases. Front Aging Neurosci 2022; 14: 815347.
[45] Cherry JD, Olschowka JA and O'Banion MK. Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. J Neuroinflammation 2014; 11: 98.
[46] Kwon HS and Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. Transl Neurodegener 2020; 9: 42.
[47] Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL and Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006; 441: 235-238.
[48] Danikowski KM, Jayaraman S and Prabhakar BS. Regulatory T cells in multiple sclerosis and myasthenia gravis. J Neuroinflammation 2017; 14: 117.
[49] Coates JR and Jeffery ND. Perspectives on meningoencephalomyelitis of unknown origin. Vet Clin North Am Small Anim Pract 2014; 44: 1157-85.
[50] Gonçalves R, De Decker S, Walmsley G, Butterfield S and Maddox TW. Inflammatory disease affecting the central nervous system in dogs: a retrospective study in England (2010-2019). Front Vet Sci 2022; 8: 819945.
[51] Granger N, Smith PM and Jeffery ND. Clinical findings and treatment of non-infectious meningoencephalomyelitis in dogs: a systematic review of 457 published cases from 1962 to 2008. Vet J 2010; 184: 290-297.
[52] Gonçalves R, De Decker S, Walmsley G and Maddox TW. Prognosis in meningoencephalitis of unknown origin in dogs: risk factors associated with survival, clinical relapse, and long-term disability. J Vet Intern Med 2024; 38: 1583-1590.
[53] Jeffery N and Granger N. New insights into the treatment of meningoencephalomyelitis of unknown origin since 2009: a review of 671 cases. Front Vet Sci 2023; 10: 1114798.
[54] Church ME, Ceja G, McGeehan M, Miller MC, Farias P, Sánchez MD, Swain GP, Assenmacher CA, Stopa EG, Vite CH, Bar-Or A and Alvarez JI. Meningeal B cell clusters correlate with submeningeal pathology in a natural model of multiple sclerosis. J Immunol 2021; 207: 44-54.
[55] Adamo PF, Adams WM and Steinberg H. Granulomatous meningoencephalomyelitis in dogs. Compend Contin Educ Vet 2007; 29: 678-90.
[56] Barnes Heller HL, Granick MN, Pinkerton ME and Keuler NS. Case-control study of risk factors for granulomatous meningoencephalomyelitis in dogs. J Am Vet Med Assoc 2019; 254: 822-825.
[57] Benito MB, Fuentes MP, Manso-Diaz G, Madonado BS and Diaz CP. Clinical signs, diagnostic imaging and histopathology in a dog with granulomatous meningoencephalitis manifested as a polyneuropathy. Vet Rec Case Rep 2022; 10: e324.
[58] Andersen-Ranberg E, Berendt M and Gredal H. Biomarkers of non-infectious inflammatory CNS diseases in dogs - where are we now? Part I: Meningoencephalitis of unknown origin. Vet J 2021; 273: 105678.
[59] Park ES, Uchida K and Nakayama H. Th1-, Th2-, and Th17-related cytokine and chemokine receptor mRNA and protein expression in the brain tissues, T cells, and macrophages of dogs with necrotizing and granulomatous meningoencephalitis. Vet Pathol 2013; 50: 1127-1134.
[60] Voo KS, Wang YH, Santori FR, Boggiano C, Wang YH, Arima K, Bover L, Hanabuchi S, Khalili J, Marinova E, Zheng B, Littman DR and Liu YJ. Identification of IL-17-producing FOXP3+ regulatory T cells in humans. Proc Natl Acad Sci U S A 2009; 106: 4793-8.
[61] Lee GR. The balance of Th17 versus treg cells in autoimmunity. Int J Mol Sci 2018; 19: 730.
[62] Müller M, Carter SL, Hofer MJ, Manders P, Getts DR, Getts MT, Dreykluft A, Lu B, Gerard C, King NJ and Campbell IL. CXCR3 signaling reduces the severity of experimental autoimmune encephalomyelitis by controlling the parenchymal distribution of effector and regulatory T cells in the central nervous system. J Immunol 2007; 179: 2774-2786.
[63] Verreycken J, Baeten P and Broux B. Regulatory T cell therapy for multiple sclerosis: breaching (blood-brain) barriers. Hum Vaccin Immunother 2022; 18: 2153534.
[64] Tokunaga R, Zhang W, Naseem M, Puccini A, Berger MD, Soni S, McSkane M, Baba H and Lenz HJ. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - a target for novel cancer therapy. Cancer Treat Rev 2018; 63: 40-47.
[65] Uchida K, Park E, Tsuboi M, Chambers JK and Nakayama H. Pathological and immunological features of canine necrotising meningoencephalitis and granulomatous meningoencephalitis. Vet J 2016; 213: 72-77.
[66] Ponomarev ED, Maresz K, Tan Y and Dittel BN. CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in mi-

croglial cells. J Neurosci 2007; 27: 10714-10721.
[67] Grunwald C, Krętowska-Grunwald A, Adamska-Patruno E, Kochanowicz J, Kułakowska A and Chorąży M. The role of selected interleukins in the development and progression of multiple sclerosis-a systematic review. Int J Mol Sci 2024; 25: 2589.
[68] Wang XX and Chen T. Meta-analysis of the association of IL2RA polymorphisms rs2104286 and rs12722489 with multiple sclerosis risk. Immunol Invest 2018; 47: 431-442.
[69] Hulshof S, Montagne L, De Groot CJA and Van Der Valk P. Cellular localization and expression patterns of interleukin-10, interleukin-4, and their receptors in multiple sclerosis lesions. Glia 2002; 38: 24-35.
[70] Qiu LJ, Ni J, Cen H, Wen PF, Zhang M, Liang Y, Pan HF, Mao C and Ye DQ. Relationship between the IL-4 gene promoter -590C/T (rs2243250) polymorphism and susceptibility to autoimmune diseases: a meta-analysis. J Eur Acad Dermatol Venereol 2015; 29: 48-55.
[71] Dewey CW, Davies ES, Xie H and Wakshlag JJ. Canine cognitive dysfunction: pathophysiology, diagnosis, and treatment. Vet Clin North Am Small Anim Pract 2019; 49: 477-499.
[72] Wolters FJ, Koudstaal PJ, Hofman A, van Duijn CM and Ikram MA. Serum apolipoprotein E is associated with long-term risk of Alzheimer's disease: The Rotterdam study. Neurosci Lett 2016; 617: 139-142.
[73] Yang HD, Kim DH, Lee SB and Young LD. History of Alzheimer's Disease. Dement Neurocognitive Disord 2016; 15: 115-121.
[74] 2024 Alzheimer's disease facts and figures. Alzheimers Dement 2024; 20: 3708-3821.
[75] Fast R, Schütt T, Toft N, Møller A and Berendt M. An observational study with long-term follow-up of canine cognitive dysfunction: clinical characteristics, survival, and risk factors. J Vet Intern Med 2013; 27: 822-829.
[76] Head E. Neurobiology of the aging dog. Age 2011; 33: 485-496.
[77] Sarasa M and Pesini P. Natural non-trasgenic animal models for research in Alzheimer's disease. Curr Alzheimer Res 2009; 6: 171-178.
[78] Panek WK, Gruen ME, Murdoch DM, Marek RD, Stachel AF, Mowat FM, Saker KE and Olby NJ. Plasma neurofilament light chain as a translational biomarker of aging and neurodegeneration in dogs. Mol Neurobiol 2020; 57: 3143-3149.
[79] Wu CH, Pan XS, Su LY and Yang SY. Plasma neurofilament light chains as blood-based biomarkers for early diagnosis of canine cognitive dysfunction syndrome. Int J Mol Sci 2023; 24: 13771.
[80] Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM and Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. Alzheimers Dement (N Y) 2018; 4: 575-590.
[81] Hickman SE, Allison EK and El Khoury J. Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. J Neurosci 2008; 28: 8354-8360.
[82] Krabbe G, Halle A, Matyash V, Rinnenthal JL, Eom GD, Bernhardt U, Miller KR, Prokop S, Kettenmann H and Heppner FL. Functional impairment of microglia coincides with Beta-amyloid deposition in mice with Alzheimer-like pathology. PLoS One 2013; 8: e60921.
[83] Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D and Yednock T. Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat Med 2000; 6: 916-919.
[84] Guillot-Sestier MV, Doty KR, Gate D, Rodriguez J Jr, Leung BP, Rezai-Zadeh K and Town T. Il10 deficiency rebalances innate immunity to mitigate Alzheimer-like pathology. Neuron 2015; 85: 534-548.
[85] Juraskova B, Andrys C, Holmerova I, Solichova D, Hrnciarikova D, Vankova H, Vasatko T and Krejsek J. Transforming growth factor beta and soluble endoglin in the healthy senior and in Alzheimer's disease patients. J Nutr Health Aging 2010; 14: 758-761.
[86] Mocali A, Cedrola S, Della Malva N, Bontempelli M, Mitidieri VA, Bavazzano A, Comolli R, Paoletti F and La Porta CA. Increased plasma levels of soluble CD40, together with the decrease of TGF beta 1, as possible differential markers of Alzheimer disease. Exp Gerontol 2004; 39: 1555-1561.
[87] Basu A, Krady JK and Levison SW. Interleukin-1: a master regulator of neuroinflammation. J Neurosci Res 2004; 78: 151-156.
[88] Buxbaum JD, Oishi M, Chen HI, Pinkas-Kramarski R, Jaffe EA, Gandy SE and Greengard P. Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. Proc Natl Acad Sci U S A 1992; 89: 10075-10078.
[89] Liao YF, Wang BJ, Cheng HT, Kuo LH and Wolfe MS. Tumor necrosis factor-alpha, interleukin-1beta, and interferon-gamma stimulate gamma-secretase-mediated cleavage of amyloid precursor protein through a JNK-dependent

MAPK pathway. J Biol Chem 2004; 279: 49523-49532.
[90] Ledo JH, Azevedo EP, Beckman D, Ribeiro FC, Santos LE, Razolli DS, Kincheski GC, Melo HM, Bellio M, Teixeira AL, Velloso LA, Foguel D, De Felice FG and Ferreira ST. Cross talk between brain innate immunity and serotonin signaling underlies depressive-like behavior induced by alzheimer's amyloid-β oligomers in mice. J Neurosci 2016; 36: 12106-12116.
[91] Plantone D, Pardini M, Righi D, Manco C, Colombo BM and De Stefano N. The role of TNF-α in alzheimer's disease: a narrative review. Cells 2023; 13: 54.
[92] Scheller J, Chalaris A, Schmidt-Arras D and Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 2011; 1813: 878-888.
[93] Quintanilla RA, Orellana DI, González-Billault C and Maccioni RB. Interleukin-6 induces Alzheimer-type phosphorylation of tau protein by deregulating the cdk5/p35 pathway. Exp Cell Res 2004; 295: 245-257.
[94] Blum-Degen D, Müller T, Kuhn W, Gerlach M, Przuntek H and Riederer P. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of alzheimer's and de novo parkinson's disease patients. Neurosci Lett 1995; 202: 17-20.
[95] Dursun E, Gezen-Ak D, Hanağası H, Bilgiç B, Lohmann E, Ertan S, Atasoy İL, Alaylıoğlu M, Araz ÖS, Önal B, Gündüz A, Apaydın H, Kızıltan G, Ulutin T, Gürvit H and Yılmazer S. The interleukin 1 alpha, interleukin 1 beta, interleukin 6 and alpha-2-macroglobulin serum levels in patients with early or late onset Alzheimer's disease, mild cognitive impairment or Parkinson's disease. J Neuroimmunol 2015; 283: 50-57.
[96] Kiyota T, Machhi J, Lu Y, Dyavarshetty B, Nemati M, Zhang G, Mosley RL, Gelbard HA and Gendelman HE. URMC-099 facilitates amyloid-β clearance in a murine model of Alzheimer's disease. J Neuroinflammation 2018; 15: 137.
[97] Huang L, Li B, Li X, Liu G, Liu R, Guo J, Xu B, Li Y and Fang W. Significance and mechanisms of p-glycoprotein in central nervous system diseases. Curr Drug Targets 2019; 20: 1141-1155.
[98] Pugliese M, Geloso MC, Carrasco JL, Mascort J, Michetti F and Mahy N. Canine cognitive deficit correlates with diffuse plaque maturation and S100β (-) astrocytosis but not with insulin cerebrospinal fluid level. Acta Neuropathol 2006; 111: 519-528.
[99] Hines AD, McGrath S, Latham AS, Kusick B, Mulligan L, Richards ML and Moreno JA. Activated gliosis, accumulation of amyloid β, and hyperphosphorylation of tau in aging canines with and without cognitive decline. Front Aging Neurosci 2023; 15: 1128521.
[100] Dansokho C, Ait Ahmed D, Aid S, Toly-Ndour C, Chaigneau T, Calle V, Cagnard N, Holzenberger M, Piaggio E, Aucouturier P and Dorothée G. Regulatory T cells delay disease progression in Alzheimer-like pathology. Brain 2016; 139: 1237-1251.
[101] Baek H, Ye M, Kang GH, Lee C, Lee G, Choi DB, Jung J, Kim H, Lee S, Kim JS, Lee HJ, Shim I, Lee JH and Bae H. Neuroprotective effects of CD4+CD25+Foxp3+ regulatory T cells in a 3xTg-AD Alzheimer's disease model. Oncotarget 2016; 7: 69347-69357.
[102] Alves S, Churlaud G, Audrain M, Michaelsen-Preusse K, Fol R, Souchet B, Braudeau J, Korte M, Klatzmann D and Cartier N. Interleukin-2 improves amyloid pathology, synaptic failure and memory in Alzheimer's disease mice. Brain 2017; 140: 826-842.
[103] Ciccocioppo F, Lanuti P, Pierdomenico L, Simeone P, Bologna G, Ercolino E, Buttari F, Fantozzi R, Thomas A, Onofrj M, Centonze D, Miscia S and Marchisio M. The characterization of regulatory T-cell profiles in alzheimer's disease and multiple sclerosis. Sci Rep 2019; 9: 8788.
[104] Fu J, Duan J, Mo J, Xiao H, Huang Y, Chen W, Xiang S, Yang F, Chen Y and Xu S. Mild cognitive impairment patients have higher regulatory T-Cell proportions compared with alzheimer's disease-related dementia patients. Front Aging Neurosci 2020; 12: 624304.
[105] Le Page A, Garneau H, Dupuis G, Frost EH, Larbi A, Witkowski JM, Pawelec G and Fülöp T. Differential phenotypes of myeloid-derived suppressor and T regulatory cells and cytokine levels in amnestic mild cognitive impairment subjects compared to mild alzheimer diseased patients. Front Immunol 2017; 8: 783.
[106] Faridar A, Vasquez M, Thome AD, Yin Z, Xuan H, Wang JH, Wen S, Li X, Thonhoff JR, Zhao W, Zhao H, Beers DR, Wong STC, Masdeu JC and Appel SH. Ex vivo expanded human regulatory T cells modify neuroinflammation in a preclinical model of Alzheimer's disease. Acta Neuropathol Commun 2022; 10: 144.
[107] Watabe A, Fukumoto S, Komatsu T, Endo Y and Kadosawa T. Alterations of lymphocyte subpopulations in healthy dogs with aging and in dogs with cancer. Vet Immunol Immunopathol 2011; 142: 189-200.
[108] Withers SS, Moore PF, Chang H, Choi JW, McSorley SJ, Kent MS, Monjazeb AM, Canter RJ, Murphy WJ, Sparger EE and Rebhun RB. Multicolor flow cytometry for evaluating age-related changes in memory lymphocyte subsets in dogs. Dev Comp Immunol 2018; 87: 64-74.
[109] Yang H, Park SY, Baek H, Lee C, Chung G, Liu X, Lee JH, Kim B, Kwon M, Choi H, Kim HJ, Kim JY, Kim Y, Lee YS, Lee G, Kim SK, Kim JS, Chang YT, Jung WS, Kim KH and Bae H. Adoptive ther-

apy with amyloid-β specific regulatory T cells alleviates Alzheimer's disease. Theranostics 2022; 12: 7668-7680.
[110] Chai AB, Leung GKF, Callaghan R and Gelissen IC. P-glycoprotein: a role in the export of amyloid-β in Alzheimer's disease? FEBS J 2020; 287: 612-625.
[111] Kooij G, van Horssen J, de Lange EC, Reijerkerk A, van der Pol SM, van Het Hof B, Drexhage J, Vennegoor A, Killestein J, Scheffer G, Oerlemans R, Scheper R, van der Valk P, Dijkstra CD and de Vries HE. T lymphocytes impair P-glycoprotein function during neuroinflammation. J Autoimmun 2010; 34: 416-425.
[112] Pekcec A, Schneider EL, Baumgärtner W, Stein VM, Tipold A and Potschka H. Age-dependent decline of blood-brain barrier P-glycoprotein expression in the canine brain. Neurobiol Aging 2011; 32: 1477-1485.
[113] Tamimi AF and Juweid M. Epidemiology and Outcome of Glioblastoma. In: De Vleeschouwer S, editor. Glioblastoma. Brisbane (AU): Codon Publications; 2017.
[114] Elguindy M, Young JS, Mondal I, Lu RO and Ho WS. Glioma-immune cell crosstalk in tumor progression. Cancers 2024; 16: 308.
[115] Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, Herndon JE 2nd, Bigner DD, Dranoff G and Sampson JH. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer Res 2006; 66: 3294-3302.
[116] Wainwright DA, Dey M, Chang A and Lesniak MS. Targeting tregs in malignant brain cancer: overcoming IDO. Front Immunol 2013; 4: 116.
[117] Komohara Y, Horlad H, Ohnishi K, Fujiwara Y, Bai B, Nakagawa T, Suzu S, Nakamura H, Kuratsu J and Takeya M. Importance of direct macrophage-tumor cell interaction on progression of human glioma. Cancer Sci 2012; 103: 2165-2172.
[118] Ye XZ, Xu SL, Xin YH, Yu SC, Ping YF, Chen L, Xiao HL, Wang B, Yi L, Wang QL, Jiang XF, Yang L, Zhang P, Qian C, Cui YH, Zhang X and Bian XW. Tumor-associated microglia/macrophages enhance the invasion of glioma stem-like cells via TGF-β1 signaling pathway. J Immunol 2012; 189: 444-453.
[119] Boyd NH, Tran AN, Bernstock JD, Etminan T, Jones AB, Gillespie GY, Friedman GK and Hjelmeland AB. Glioma stem cells and their roles within the hypoxic tumor microenvironment. Theranostics 2021; 11: 665-683.
[120] Maes W, Verschuere T, Van Hoylandt A, Boon L and Van Gool S. Depletion of regulatory T cells in a mouse experimental glioma model through anti-CD25 treatment results in the infiltration of non-immunosuppressive myeloid cells in the brain. Clin Dev Immunol 2013; 2013: 952469.
[121] Panek WK, Toedebusch RG, Mclaughlin BE, Dickinson PJ, Van Dyke JE, Woolard KD, Berens ME, Lesniak MS, Sturges BK, Vernau KM, Li C, Miska J and Toedebusch CM. The CCL2-CCR4 axis promotes Regulatory T cell trafficking to canine glioma tissues. J Neurooncol 2024; 169: 647-658.
[122] Candolfi M, Curtin JF, Nichols WS, Muhammad AG, King GD, Pluhar GE, McNiel EA, Ohlfest JR, Freese AB, Moore PF, Lerner J, Lowenstein PR and Castro MG. Intracranial glioblastoma models in preclinical neuro-oncology: neuropathological characterization and tumor progression. J Neurooncol 2007; 85: 133-148.
[123] Pi Castro D, José-López R, Fernández Flores F, Rabanal Prados RM, Mandara MT, Arús C and Pumarola Batlle M. Expression of FOXP3 in canine gliomas: immunohistochemical study of tumor-infiltrating regulatory lymphocytes. J Neuropathol Exp Neurol 2020; 79: 184-193.
[124] Filley A, Henriquez M, Bhowmik T, Tewari BN, Rao X, Wan J, Miller MA, Liu Y, Bentley RT and Dey M. Immunologic and gene expression profiles of spontaneous canine oligodendrogliomas. J Neurooncol 2018; 137: 469-479.
[125] Krane GA, O'Dea CA, Malarkey DE, Miller AD, Miller CR, Tokarz DA, Jensen HL, Janardhan KS, Shockley KR, Flagler N, Rainess BA and Mariani CL. Immunohistochemical evaluation of immune cell infiltration in canine gliomas. Vet Pathol 2021; 58: 952-963.
[126] Amin SB, Anderson KJ, Boudreau CE, Martinez-Ledesma E, Kocakavuk E, Johnson KC, Barthel FP, Varn FS, Kassab C, Ling X, Kim H, Barter M, Lau CC, Ngan CY, Chapman M, Koehler JW, Long JP, Miller AD, Miller CR, Porter BF, Rissi DR, Mazcko C, LeBlanc AK, Dickinson PJ, Packer RA, Taylor AR, Rossmeisl JH Jr, Woolard KD, Heimberger AB, Levine JM and Verhaak RGW. Comparative molecular life history of spontaneous canine and human gliomas. Cancer Cell 2020; 37: 243-257, e7.
[127] Sayour EJ, McLendon P, McLendon R, De Leon G, Reynolds R, Kresak J, Sampson JH and Mitchell DA. Increased proportion of FoxP3+ regulatory T cells in tumor infiltrating lymphocytes is associated with tumor recurrence and reduced survival in patients with glioblastoma. Cancer Immunol Immunother 2015; 64: 419-427.
[128] Toedebusch R, Grodzki AC, Dickinson PJ, Woolard K, Vinson N, Sturges B, Snyder J, Li CF, Nagasaka O, Consales B, Vernau K, Knipe M, Murthy V, Lein PJ and Toedebusch CM. Glioma-associated microglia/macrophages augment tumorigenicity in canine astrocytoma, a natu-

rally occurring model of human glioma. Neurooncol Adv 2021; 3: vdab062.
[129] Chang AL, Miska J, Wainwright DA, Dey M, Rivetta CV, Yu D, Kanojia D, Pituch KC, Qiao J, Pytel P, Han Y, Wu M, Zhang L, Horbinski CM, Ahmed AU and Lesniak MS. CCL2 produced by the glioma microenvironment is essential for the recruitment of regulatory T cells and myeloid-derived suppressor cells. Cancer Res 2016; 76: 5671-5682.
[130] Panek WK, Pituch KC, Miska J, Kim JW, Rashidi A, Kanojia D, Lopez-Rosas A, Han Y, Yu D, Chang CL, Kane JR, Zhang P, Cordero A and Lesniak MS. Local application of autologous platelet-Rich fibrin patch (PRF-P) suppresses regulatory T cell recruitment in a murine glioma model. Mol Neurobiol 2019; 56: 5032-5040.
[131] Wyatt S, Llabres-Diaz F, Lee CY and Beltran E. Early CT in dogs following traumatic brain injury has limited value in predicting short-term prognosis. Vet Radiol Ultrasound 2021; 62: 181-189.
[132] Maeda S, Motegi T, Iio A, Kaji K, Goto-Koshino Y, Eto S, Ikeda N, Nakagawa T, Nishimura R, Yonezawa T and Momoi Y. Anti-CCR4 treatment depletes regulatory T cells and leads to clinical activity in a canine model of advanced prostate cancer. J Immunother Cancer 2022; 10: e003731.
[133] Debinski W, Dickinson P, Rossmeisl JH, Robertson J and Gibo DM. New agents for targeting of IL-13RA2 expressed in primary human and canine brain tumors. PLoS One 2013; 8: e77719.
[134] Boozer LB, Davis TW, Borst LB, Zseltvay KM, Olby NJ and Mariani CL. Characterization of immune cell infiltration into canine intracranial meningiomas. Vet Pathol 2012; 49: 784-795.
[135] Saito R, Chambers JK and Uchida K. The expression of platelet-derived growth factor and its receptor in canine and feline meningiomas. J Vet Med Sci 2023; 85: 1057-1062.
[136] Harmanci AS, Boudreau B, Lau S, Hosseingholi Nouri S, Mandel JJ, Lu HC, Harmanci AO, Klisch TJ, Levine JM and Patel AJ. Aggressive human MenG C meningiomas have a molecular counterpart in canines. Acta Neuropathol 2024; 147: 42.
[137] Zakimi N, Mazcko CN, Toedebusch C, Tawa G, Woolard K, LeBlanc AK, Dickinson PJ and Raleigh DR. Canine meningiomas are comprised of 3 DNA methylation groups that resemble the molecular characteristics of human meningiomas. Acta Neuropathol 2024; 147: 43.
[138] Boyle-Walsh E, Birch M, Gallagher JA, Speirs V, White MC, Shenkin A and Fraser WD. RT-PCR detection of cytokine transcripts in a series of cultured human meningiomas. J Pathol 1996; 178: 442-446.
[139] Todo T, Adams EF, Rafferty B, Fahlbusch R, Dingermann T and Werner H. Secretion of interleukin-6 by human meningioma cells: possible autocrine inhibitory regulation of neoplastic cell growth. J Neurosurg 1994; 81: 394-401.
[140] Al-Nadaf S, Platt SR, Kent M, Northrup N and Howerth EW. Minimal interleukin expression in canine intracranial meningiomas. Vet Rec 2015; 177: 75.
[141] Goswami TK, Singh M, Dhawan M, Mitra S, Emran TB, Rabaan AA, Mutair AA, Alawi ZA, Alhumaid S and Dhama K. Regulatory T cells (Tregs) and their therapeutic potential against autoimmune disorders - Advances and challenges. Hum Vaccines Immunother 2022; 18: 2035117.
[142] Cummings J, Zhou Y, Lee G, Zhong K, Fonseca J and Cheng F. Alzheimer's disease drug development pipeline: 2024. Alzheimers Dement (N Y) 2024; 10: e12465.
[143] Mason NJ, Gnanandarajah JS, Engiles JB, Gray F, Laughlin D, Gaurnier-Hausser A, Wallecha A, Huebner M and Paterson Y. Immunotherapy with a HER2-targeting listeria induces HER2-specific immunity and demonstrates potential therapeutic effects in a phase i trial in canine osteosarcoma. Clin Cancer Res 2016; 22: 4380-4390.
[144] Olin MR, Ampudia-Mesias E, Pennell CA, Sarver A, Chen CC, Moertel CL, Hunt MA and Pluhar GE. Treatment combining CD200 immune checkpoint inhibitor and tumor-lysate vaccination after surgery for pet dogs with high-grade glioma. Cancers (Basel) 2019; 11: 137.
[145] LeBlanc A, Mazcko CN, Mason NJ, Chambers MR, Brockington DM, Pluhar GE and Naik S. Comparative oncology in action: vignettes on immunotherapy development. Vet Oncol 2025; 2: 5.
[146] Quigley M, McKenna C and Webb TL. Best practices for recruitment in veterinary clinical trials. Front Vet Sci 2024; 11: 1418747.
[147] Thumser-Henner P, Nytko KJ and Rohrer Bley C. Mutations of BRCA2 in canine mammary tumors and their targeting potential in clinical therapy. BMC Vet Res 2020; 16: 30.
[148] Zink C, Delgado MM and Stella JL. Vasectomy and ovary-sparing spay in dogs: comparison of health and behavior outcomes with gonadectomized and sexually intact dogs. J Am Vet Med Assoc 2023; 261: 366-374.
[149] Oyama MA, Ellenberg SS and Shaw PA. Clinical trials in veterinary medicine: a new era brings new challenges. J Vet Intern Med 2017; 31: 970-978.
[150] Lowther DE and Hafler DA. Regulatory T cells in the central nervous system. Immunol Rev 2012; 248: 156-169.