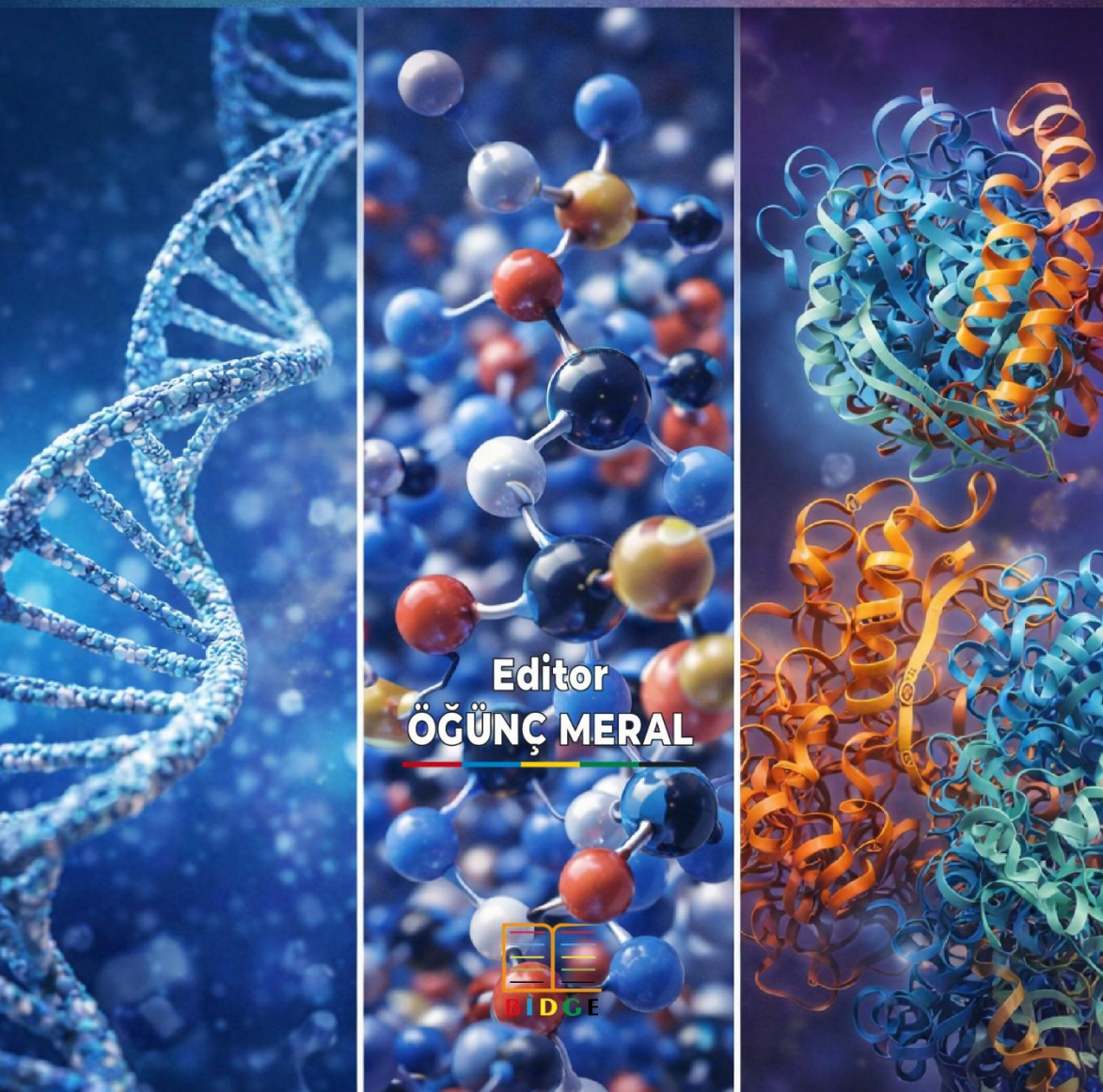
MOLECULAR LANDSCAPES OF BIOCHEMISTRY



Editor
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PREFACE

A living organism is not only a sum of the reactions occurring within it. A living organism is a complex system. Within this complex system, the balance between molecular pathways defines the main distinction between health and disease. The book, "Molecular Landscapes of Biochemistry," resulted from the belief that understanding disease mechanisms requires a detailed investigation of these complex biochemical processes.

Each chapter of this book addresses extensively one area of current biochemistry research, while emphasizing the elements that link all of these areas. On the one hand, this book is based on the idea that biochemical abnormalities are the core reason for which pathogenesis occurs and that the knowledge of the molecular mechanism is the key to all new treatment strategies. This book has been prepared for students, researchers, and clinicians who would like to explore the link between basic biochemistry and complex diseases.

We invite you to join us in exploring these molecular landscapes.

Assoc. Prof. Dr. Öğünç MERAL
Editor

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EXPERIMENTAL ALZHEIMER'S DISEASE MODELS IN LABORATORY ANIMALS

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Introduction

Experimental models of Alzheimer's disease have been created in order to assess possible treatment strategies, delay the illness's course, and learn more about the mechanisms behind its start. Transgenic and non-transgenic models are the two basic categories into which these models can be divided. Transgenic models create genetically changed creatures by introducing genetic mutations into experimental animals like mice or rats. Many methods, including as chemical agents and surgical techniques, are used in non-transgenic models. Simulating Alzheimer's disease in people and facilitating experimental uses like drug testing are the main goals of developing experimental Alzheimer's disease models in animals. It is crucial to comprehend the pathological alterations brought on by Alzheimer's disease and to closely adapt these

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alterations to animal models in order to create suitable and trustworthy experimental Alzheimer's models that faithfully replicate the illness.

Alzheimer's Disease

The most frequent cause of dementia, a neurological disorder that impairs mental capacities including language, orientation, and personality, is Alzheimer's disease. It is described as a deadly degenerative dementia that starts with minor memory loss and gradually progresses to total loss of both cognitive and motor skills. The hippocampus and cerebral cortex are intimately linked to Alzheimer's disease. The formation of amyloid plaques and neurofibrillary tangles in particular areas of the cerebral cortex is the main cause of the disease. Cognitive processes like memory, language, thought, and decision-making are controlled by these impacted cortical areas. The buildup of these diseased structures causes neuronal damage and eventually neuronal death as the disease advances. The loss of neurons is accompanied by a marked decline in cognitive functions (Busciglio et al., 1997).

There are two types of Alzheimer's disease: sporadic (SAD) and familial (FAD). Compared to the sporadic variety, familial, genetically inherited Alzheimer's disease is less common and usually manifests earlier (Duara et al., 1993).

A primary cause of reliance, disability, and mortality, dementia is a progressive, acquired cognitive condition that is severe enough to interfere with day-to-day activities. Alzheimer's disease is the most common form of dementia, making up between 50 and 75 percent of all dementia cases. It primarily affects older adults, with its frequency about doubling every five years after age 65. According to current estimates, 44 million people worldwide suffer from dementia; as the world's population ages, this figure is predicted to more than treble by 2050. Additionally, the annual

expense of dementia in the US alone is estimated to be more than 600 billion USD (Prince et al., 2014; Prince et al., 2009). In England and Wales, dementia is among the leading causes of death, accounting for 11.6% of all recorded deaths in 2015 (Lane et al., 2017).

Alzheimer's disease has been mainly linked to four genes: APOE, APP, PSEN1, and PSEN2. Of these, only APOE is linked to sporadic inheritance; the others cause familial Alzheimer's disease (FAD) and are inherited autosomally dominantly. Mutations in the APP, Presenilin 1, and Presenilin 2 genes can result in familial Alzheimer's disease, even though the disease most frequently manifests sporadically. Between the ages of 30 and 50, familial Alzheimer's disease symptoms commonly manifest before those of sporadic Alzheimer's disease (Bateman et al., 2011; Lanoiselée et al., 2017).

It is believed that a complicated interplay between hereditary and environmental variables causes the more prevalent sporadic, late-onset form of Alzheimer's disease. Genetic factors are thought to account for about 70% of the risk for AD. One of the main genetic risk factors for sporadic Alzheimer's disease is thought to be APOE. Human APOE is a lipoprotein of 299 amino acids that is expressed in most organs, with the liver and brain showing the highest levels of expression. In humans, there are three main polymorphic isoforms of APOE: APOE2, APOE3, and APOE4. Lipids are transported, delivered, and distributed by APOE, a crucial part of lipoprotein complexes that control lipid metabolism. It uses APOE receptors and associated proteins to transport lipids from one tissue or cell type to another (Lane et al., 2017; Saunders et al., 2000).

About 50–90% of people have APOE3, 5–35% have APOE4, and 1–5% have APOE2 (Mahley et al., 2000). APOE4 is the strongest genetic risk factor in Alzheimer's disease risk stratification.

A single copy of the APOE4 allele increases the risk of late-onset Alzheimer's disease by around three times, while homozygosity (carrying two copies) increases the risk by nearly twelve times. Additionally, in late-onset individuals, the presence of one or two APOE4 alleles is linked to an earlier age of illness onset, usually 10–20 years earlier than in non-carriers. On the other hand, people with the APOE2 allele are less likely to get late-onset Alzheimer's (Verghese et al., 2011). Despite its strong association with increased risk, APOE4 alone is not sufficient to cause Alzheimer's disease; therefore, APOE4 polymorphism cannot be used as a sole diagnostic marker (Meyer et al., 1998; Zhao et al., 2018).

The Pathogenesis of Alzheimer's Disease

Extracellular amyloid-β accumulation, intracellular tau accumulation, neuronal and synaptic loss, brain shrinkage, and inflammation are the pathogenic features of the disease. The pathophysiology of Alzheimer's disease has been explained by two major theories. One of these theories is about the buildup of amyloid, while the other is about the tau protein's hyperphosphorylation. The amyloid accumulation theory states that mutations in the amyloid precursor protein (APP) cause aberrant cleavage of APP, which leads to an excess of amyloid-β. This extra amyloid-β eventually creates amyloid plaques, which kill neurons. When this theory was first put forth, it was believed that the toxic consequences of accumulating amyloid caused neuronal malfunction and death. Research on more specific changes in Aβ processing, such as the cleavage of APP into Aβ peptides (Aβ1–40 and Aβ1–42) and the significance of Aβ oligomers—small aggregates made up of two to twelve peptides—has increased as our understanding of the pathophysiology of Alzheimer's disease has grown. Tau hyperphosphorylation is thought to be caused by mutations in the Microtubule-Associated Protein Tau gene, which codes for the tau protein. Microtubules are essential for intracellular

transport, and tau is in charge of keeping them stable. Tau guarantees the correct operation of microtubules by attaching to them. Mutations in the MAPT gene can lead to excessive production or hyperphosphorylation of tau, resulting in the formation of neurofibrillary tangles (NFTs) (Armstrong et al., 2006).

Both familial and sporadic late-onset forms of Alzheimer's disease have different mutations. APOE (Apolipoprotein E) is important in the sporadic form. On the other hand, presenilin-1 (PSEN1) on chromosome 14, presenilin-2 (PSEN2) on chromosome 1, and APP on chromosome 21 are genes linked to the pathophysiology of familial Alzheimer's disease (FAD). While presenilin mutations increase γ-secretase activity, which increases APP processing and leads to higher formation of Aβ42 and Aβ40, changes in the APP gene cause increased amyloid synthesis and aggregation (Bekris et al., 2010). As of right now, the APP gene has 51 harmful mutations, the PSEN1 gene has 219, and the PSEN2 gene has 16 (Esquerda-Canals et al., 2017).

Amyloid precursor protein is produced more frequently when the APP gene is mutated. The presenilin-1 and presenilin-2 proteins, which are encoded by the presenilin-1 and presenilin-2 genes, control the activity of the γ-secretase complex, which cleaves a number of proteins, including APP. Amyloid-β synthesis is increased when γ-secretase activity is elevated due to mutations in these genes (Dehury et al., 2019).

The amyloidogenic and non-amyloidogenic pathways are the two ways that amyloid precursor protein (APP) is cleaved under normal circumstances. The non-amyloidogenic route does not produce amyloid-β; instead, α-secretase cleaves APP to produce soluble APPα (sAPPα) and a peptide fragment called C83. On the other hand, β-secretase cleaves APP in the amyloidogenic pathway, producing soluble APPβ (sAPPβ) and a shorter peptide fragment

known as C99. After cleaving C99, γ-secretase releases the amyloid-β peptide into the extracellular space, where it builds up. Amyloid-β peptides of various lengths, mainly Aβ40 and Aβ42, can be produced by γ-secretase cleavage. While Aβ42 is longer and more closely linked to the pathogenesis of Alzheimer's disease, Aβ40 is the more prevalent and shorter variant (Orobets et al., 2023).

Amyloid plaques are formed by the abnormal extracellular accumulation of misfolded Aβ40 and Aβ42 peptides, which are by-products generated during APP cleavage (Öztürk et al., 2009).

Experimental Alheimer's Disease Models

Researchers started looking for models that could replicate important pathological characteristics like amyloid plaque formation and tau hyperphosphorylation, as well as functional manifestations like impaired neuronal transmission and neuronal death, after the effects of Alzheimer's disease on the brain and its underlying mechanisms of development were better understood. The creation of these models is crucial for advancing our knowledge of the pathophysiology of diseases, facilitating preventative measures, delaying the advancement of diseases, and evaluating possible treatment modalities. Several techniques have been used to create these models, such as inducing the formation of amyloid plaque, creating neurofibrillary tangles (NFTs), inhibiting the release of neurotransmitters involved in cholinergic neurotransmission, and inducing neuronal death. Consequently, a variety of unique models have been created utilizing various methodological techniques. There are various approaches to categorize these models (Şahin et al., 2012).

Transgenic Alzheimer's disease models and non-transgenic Alzheimer's disease models are the two main types of experimental Alzheimer's disease models.

Transgenic Alzheimer's Disease Models

Transgenic organisms are those that have a foreign gene fragment inserted into their own genome as a transgene. The processes by which transgenic models display characteristics similar to Alzheimer's disease can be used to categorize them. Transgenic models called amyloid beta models were created to encourage the development of amyloid plaque. Tau models, on the other hand, are transgenic models where neurofibrillary tangles (NFTs) are formed as a result of hyperphosphorylation of the tau protein and its self-aggregation. Transgenic organisms are created by adding mutations in the APP, PSEN1, or PSEN2 genes to cause the production of amyloid plaque. Many transgenic models have been created for use in experiments due to variations in the kinds of mutations introduced, the techniques used to produce these changes, and the desired research objectives. Various species, including mice, rats, zebrafish, and primates, can be used in these studies. However, species other than mice and rats are rarely chosen as transgenic models due to ethical considerations, issues with animal housing and nutrition, difficulties with genetic alteration, and the length of time needed to produce progeny. Because of these factors, transgenic Alzheimer's disease research most frequently uses mice and rats. While there are rat-based models, they are much less common than mouse models, and most models of Alzheimer's disease have been developed in mice (Elçioğlu et al., 2018).

Transgenic Amyloid Beta Models

In these models, mutations in the APP gene that cause its overexpression and in the presenilin genes that increase γ-secretase–mediated cleavage cause amyloid-β formation. Various isoforms of the human APP gene are frequently employed in the majority of these models.

Tg2576 Transgenic Mouse Model

The human APP gene's 695 isoform is present in the Tg2576 transgenic mouse model, which is genetically modified to overexpress this gene under the direction of the hamster prion protein promoter. The K670N/M671L mutations, often known as the Swedish mutation, are present in this version of the human APP gene. The human APP gene is expressed at roughly five times higher levels in the Tg2576 model than in normal mice due to these mutations (Hsiao et al., 1996; Terai et al., 2001).

The Swedish mutation results from the replacement of leucine (L) for methionine (M) at position 671 and asparagine (N) for lysine (K) at position 670 in the human APP gene (Hsiao et al., 1996). These mutations boost processing through the β-secretase pathway and prevent APP cleavage through the α-secretase pathway. The pathogenesis of Alzheimer's disease is characterized by high amyloid plaque development, which is caused by a substantial rise in amyloid-β synthesis. These transgenic mice experience age-dependent memory loss linked to amyloid plaque buildup. Tg2576 mice are a good model to examine plaque-associated cognitive impairment in Alzheimer's disease because of the relationship between aging, plaque burden, and memory decline. While amyloid plaque deposition usually starts between 9 and 12 months of age in Tg2576 mice, amyloid-β production starts to rise quickly at around 6 months of age (Kawarabayashi et al., 2001). While Westerman et al. (2002) reported plaque development at roughly 11–12 months, Hsiao et al. (1996) found that amyloid plaque formation occurred at 9–12 months of age. Despite its value in simulating memory impairment, one of the Tg2576 model's primary drawbacks is the challenge of accurately identifying the beginning of cognitive decline and regulating the pace of illness progression (Westerman et al., 2002). Tg2576 mice do not exhibit any significant neuroanatomical defects at younger ages, despite having high APP

and amyloid-β levels. But as these mice get older, extensive amyloid-β deposits show up, especially in areas of the brain that are specifically impacted by Alzheimer's disease. Neurofibrillary tangles are absent in Tg2576 mice, and even in very old animals, there is no neuronal loss, which is similar to the PDAPP mouse model (Dodart et al., 2002). When combined, these results imply that many of the early neuropathological characteristics of Alzheimer's disease are present in Tg2576 transgenic mice.

In conclusion, juvenile Tg2576 mice do not exhibit notable neuroanatomical defects despite comparatively high brain levels of APP and amyloid-β. However, these transgenic mice experience increased diffuse amyloid-β and amyloid deposits as they age, especially in areas of the brain susceptible to Alzheimer's disease. The majority of these deposits are linked to dystrophic neurites and inflammatory processes. Neurofibrillary tangles do not form in Tg2576 mice, and even at advanced ages, there is no evidence of neuronal death, as shown in PDAPP mice. All of the information that is now available suggests that Tg2576 mice mimic many of the early neuropathological traits of Alzheimer's disease (Dodart et al., 2002).

Additionally, a study by Alexander et al. (2011) showed that the Tg2576 mouse model is useful for simulating both cognitive impairment and non-cognitive behavioral abnormalities, like agitation and aggression, which are commonly seen in Alzheimer's disease.

5xFAD Transgenic Mouse Model

A popular and reliable model created to study the pathophysiology of Alzheimer's disease is the 5xFAD transgenic mice model. Three APP gene mutations and two PSEN1 gene mutations are among the five point mutations present in this model. The Swedish (K670N/M671L), London (V717I), and Florida

(I716V) mutations are the names given to the three APP mutations. These mutations cause the APP gene to be overexpressed, which raises the amount of amyloid precursor protein (APP) produced. As a result, the likelihood of amyloid-β formation is boosted by the increased availability of APP. The structure of the γ-secretase complex is impacted by the two point mutations, M146L and L286V, that were inserted into the PSEN1 gene. These mutations further raise amyloid-β levels by promoting greater production of the presenilin protein, which is responsible for amyloid-β formation. Amino acid changes at particular locations within the corresponding genes are the cause of all five mutations. The Thy1 promoter, which promotes neuron-specific expression, is used to overexpress these mutant genes, leading to significant amyloid buildup (Oakley et al., 2006; Forner et al., 2021).

Alzheimer's disease-like pathology occurs quickly in this animal due to the combined impact of mutations that both increase APP synthesis and improve its cleavage. Specifically, early and strong amyloid plaque development is caused by rapid and excessive synthesis of the Aβ42 peptide. Because of this, extensive plaque deposition can be identified as early as 1.5 to 2 months of age (Eimer & Vassar, 2013). Interestingly, intraneuronal Aβ42 accumulation starts as early as 1.5 months of age, before extracellular plaque development, which happens around 2 months later, according to Oakley et al. (2006).

The 5xFAD transgenic animal has been extensively used in experiments due to the presence of five different familial Alzheimer's disease mutations and the consequent high amounts of amyloid formation. For instance, Cai et al. (2019) examined how electroacupuncture treatment affected 5xFAD mice's neuroinflammation and cognitive decline. According to their research, electroacupuncture can effectively reduce

neuroinflammation, increase cerebral metabolic activity, alleviate cognitive impairments, and modulate amyloid-β buildup.

Forner et al. (2021) conducted a thorough phenotypic assessment of the 5xFAD mouse model in a different investigation, looking at behavioral changes and neurophysiological deficits. For example, compared to age-matched control mice, 18-month-old 5xFAD mice showed decreased movement distance and velocity in the open-field test, suggesting a loss in motor function. Furthermore, age-dependent increases in microglial and astrocyte counts were found in the cortex and hippocampus of mice aged 4, 8, and 18 months, indicating increasing neuroinflammation. Age-related alterations in 5xFAD mice were thoroughly examined using a variety of behavioral, histological, and physiological tests.

Another study by Xu et al. (2022) looked at how a ketogenic diet affected Alzheimer's disease and found that it decreased neuroinflammation and cognitive impairment.

In conclusion, even a quick summary of the uses of the 5xFAD model emphasizes how crucial experimental models of Alzheimer's disease are to improving our knowledge of the disease's pathogenesis, assessing possible treatment approaches, and looking into ways to slow the disease's progression or postpone the onset of symptoms.

PDAPP Trangenic Mouse Model

Three isoforms of the human APP gene—APP695, APP751, and APP770—are employed in this transgenic model. The human APP gene has a V717F mutation, and the platelet-derived growth factor (PDGF) promoter drives the overexpression of this mutant gene. The amino acid valine (V) at position 717 is changed to phenylalanine (F) in the V717F mutation. This alteration causes the human APP gene to be expressed at a high level, which raises the

amount of amyloid-β produced. Amyloid deposition in the brains of PDAPP mice starts about 6 months of age due to increased amyloid-β levels. One of the earliest mice models of Alzheimer's disease, this model was first reported by Games et al. in 1995 (Webster et al., 2014). Because amyloid plaques observed in PDAPP mice closely resemble Aβ deposits seen in Alzheimer's disease, this model is regarded as a successful experimental representation of amyloid pathology (Games et al., 1995).

The brains of PDAPP mice have also been shown to exhibit neurodegenerative alterations. These alterations include aberrant phosphorylation of cytoskeletal components and loss of synaptic and dendritic proteins (Chen et al., 1998). Both diffuse and compact forms of the resultant plaques are visible. Neurofibrillary tangles have not yet been seen in PDAPP transgenic mice, though. Significant deficits in learning and memory activities linked to amyloid buildup in these animals have been shown by behavioral investigations. The Morris water maze (MWM) test has been used to thoroughly assess spatial working memory in PDAPP mice. This ability allows people to recall the locations, movements, and spatial relationships of objects, which is crucial for everyday tasks like navigation, recalling object locations, and estimating distances. Spatial working memory deficits start to show up as early as 4 months of age and continue for the duration of the model's life. One especially notable aspect of the PDAPP model is that impairments in spatial working memory occur before detectable amyloid accumulation (Webster et al., 2014).

Recognition memory deficits don't show up until about six months of age. The Novel Object Recognition (NOR) task, which gauges an animal's preference for a novel object over a familiar one, has been used to evaluate recognition memory. Research has demonstrated that cognitive deficiencies in recognition memory

exist at six months of age, although they are not as severe as deficiencies in working memory (Webster et al., 2014).

Because of its strong plaque formation and prominence as one of the first models of Alzheimer's disease, the PDAPP model is widely utilized for vaccination and immunotherapy research (Johnson-Wood et al., 1997). This model clearly expresses several important characteristics of Alzheimer's disease in a way that is reliant on age and gene expression, even though it does not reproduce all clinical hallmarks of the illness. As with all current models, PDAPP mice are an appropriate transgenic model for assessing therapeutic interventions that target different stages of neurodegeneration linked to cognitive impairment in Alzheimer's disease. However, it should be noted that this model does not fully capture the complexity of Alzheimer's disease pathology (Tanzi et al., 1995).

In conclusion, the PDAPP mouse model is a useful tool for studying the development of strong amyloid plaque, especially amyloid pathology and the early cognitive abnormalities that are linked to it. Among this model's most prominent characteristics are the early start of spatial working memory impairments and the development of cognitive abnormalities before amyloid deposition. PDAPP mice are particularly favored in vaccine-based treatment studies and are frequently used to clarify the pathophysiology of Alzheimer's disease and assess possible therapeutic approaches (Webster et al., 2014).

APP23 Transgenic Mouse Model

The human APP gene with the Swedish double mutation is overexpressed in the APP23 transgenic mouse strain. Amino acid changes at sites 670 and 671 of the human APP gene result in the Swedish mutation (K670N, M671L). The goal of this model is to induce significant levels of amyloid-β accumulation by

overexpressing the Swedish-mutant form of the human APP gene utilizing the Thy1.2 promoter, which induces robust expression in the brain and immune cells. In APP23 mice, amyloid plaque development starts about six months of age, and the plaque burden gradually rises with age in tandem with the pathophysiology of Alzheimer's disease. Amyloid deposition is also seen in cerebral blood vessels after plaque formation at about six months, signifying the onset of cerebral amyloid angiopathy. It has been observed that female mice develop plaque buildup more quickly than male mice. As people age, additional pathological characteristics linked to Alzheimer's disease are also seen, such as inflammatory reactions, neuronal and synaptic degradation, and neuronal loss (Sturchler-Pierrat & Staufenbiel, 2000).

The APP23 mouse has been extensively employed in both mechanistic and therapeutic research as a reliable model for the development of amyloid plaque. For instance, Wilhelmus et al. (2022) created APP23 mice with the TG2 gene deleted in order to examine the function of tissue transglutaminase (TG2) in Aβ pathogenesis. By using immunohistochemical analyses, they showed that APP23 mice lacking TG2 had significantly less amyloid-β buildup than conventional APP23 mice. TG2 may contribute to amyloid-β pathology and be a possible therapeutic target in Alzheimer's disease, as evidenced by statistically significant reductions in vascular amyloid deposits and senile plaques in the cortex.

The Barnes maze test has been used to study the effects of age-dependent amyloid plaque buildup on spatial learning and memory in APP23 mice. The Barnes maze evaluates a mouse's capacity to find an escape tunnel among several holes on a circular platform. In a study using 12-month-old APP23 mice, animals with amyloid plaque accumulation made more mistakes and took a lot longer to find the escape tunnel than age-matched control mice.

APP23 mice also demonstrated delayed performance during the reverse learning phase, which assesses cognitive flexibility by relocating the escape tunnel (Prut et al., 2007). These results suggest that amyloid buildup in APP23 mice impairs cognitive abilities like spatial memory and learning. Early-stage APP23 mice exhibit increased aggressiveness and sleep-wake cycle disruptions in studies looking at behavioral and psychological aspects of Alzheimer's disease, while advancing neuropathology is linked to social disengagement and behaviors resembling depression (Kosel et al., 2020).

Studies evaluating possible treatment approaches have also used APP23 mice. For example, Lucchetti et al. (2019) employed APP23 mice to measure doxycycline's brain penetration and plasma and brain concentrations after single and repeated treatment regimens. These findings demonstrate that the APP23 model, like other models of Alzheimer's disease, has been extensively used in a variety of experimental settings and has been helpful in determining the pathophysiology of the disease and assessing pharmacological therapies related to Alzheimer's disease.

J20 Transgenic Mouse Model

The Swedish and Indiana variants of the human APP gene are present in the J20 model, a transgenic mouse model. These mutations cause the APP gene to be overexpressed, which raises the amount of amyloid precursor protein (APP). Thus, more γ-secretase-mediated APP cleavage results in more amyloid-β being produced. In this animal, intracellular amyloid-β buildup is first seen at two months of age. Vascular amyloid-β deposition starts to show up about three months of age due to aging and rising amyloid burden. At this age, albumin leakage from cerebral blood vessels has been seen in J20 mice's brains, suggesting that the blood–brain barrier has been disrupted (Shibly et al., 2022).

Studies analyzing different approaches to measuring cognitive performance have also benefited from the J20 model. The cheese board test has been studied as a possible substitute when the Morris water maze (MWM) is inappropriate. In the cheese board test, which evaluates cognitive abilities including spatial learning and memory, J20 mice did poorly. These results support the use of the cheese board test as an alternative to the Morris water maze and show cognitive impairment in J20 mice (Karl et al., 2012). This work demonstrates how behavioral tests used to evaluate cognitive function can be assessed for efficacy and interchangeability by looking at whether they successfully elicit the desired behavioral responses in particular mouse models (Lanooij et al., 2023(b))

The impact of the social environment on the pathology of Alzheimer's disease has also been studied using the J20 model. The effects of individual versus group housing on plaque formation in J20 mice were investigated in a study by Lanooij et al. (2023a). In contrast to group-housed mice, individually housed J20 animals showed less hippocampus plaque formation. Other pathogenic characteristics like tau hyperphosphorylation and neuronal degeneration were not significantly impacted by housing conditions.

For the purpose of studying Alzheimer's disease, many different transgenic mouse models with mutations in the APP and PSEN genes have been created in addition to the J20 model. These consist of the H6, C9, CRND8, APPDutch, and PSAPP models, among others. Every model has unique benefits and drawbacks. For instance, plaque distribution may be more localized in some models and more extensive in others; plaque load may be larger or lower depending on the model; and amyloid plaque development may start earlier in some models and later in others. Carefully choosing the best model is crucial for answering certain research questions in light of these inter-model variations (Elder et al., 2010).

Transgenic Tau Models

The MAPT gene encodes the intracellular tau protein, which is involved in the development of the cellular cytoskeleton. A cell's cytoskeletal framework, or internal scaffold, determines its form. Tau is a microtubule-associated protein that stabilizes microtubules in neurons under normal circumstances. According to Pîrşcoveanu et al. (2017), normal tau protein in the adult human brain has two to three moles of phosphate per mole of tau protein. However, tau experiences hyperphosphorylation in neurodegenerative illnesses like Alzheimer's disease, which results in the development of aggregates of hyperphosphorylated tau called neurofibrillary tangles (NFTs). A collection of neurological conditions known as tauopathies are caused by these pathological changes in tau. Hyperphosphorylated tau can impair normal tau function, resulting in disruption of the cellular cytoskeleton, and the accumulation of neurofibrillary tangles may further interfere with neuronal signaling (Jouanne et al., 2017).

JNPL3 Transgenic Mouse Model

The 0N4R isoform of human tau protein and the P301L mutation are present in the JNPL3 transgenic mouse model. In this model, the mouse prion protein (Prnp) promoter drives the expression of the mutant tau protein (Denk et al., 2009). Tau protein buildup and the development of pathogenic tau species, which are indicative of Alzheimer's disease, are clearly visible in this model. Different pathogenic tau species are produced when hyperphosphorylated tau proteins inappropriately combine. Neurofibrillary tangles, which are seen in Alzheimer's disease, start to form in the JNPL3 model about 4.5 months of age. These neurofibrillary tangles are primarily found in the spinal cord, hindbrain, and diencephalon as the animals get older (Vitale et al., 2018). In order to assess different treatment approaches for

Alzheimer's disease and other tauopathy-related disorders, the JNPL3 mouse model has also been extensively utilized. For instance, direct intracerebral injection of the scFv-MC1 antibody decreased the amounts of both soluble and insoluble tau protein in the mouse brain in a study by Vitale et al. (2018). This study shows that experimental models of Alzheimer's disease are useful instruments for evaluating possible treatment strategies.

In conclusion, these findings suggest that the JNPL3 transgenic mouse model is a valuable resource for comprehending tau pathology, a crucial pathological feature of Alzheimer's disease, and for evaluating possible treatment approaches that target this pathology. The discovery of successful treatments for Alzheimer's disease depends heavily on research using various therapeutic approaches in this model (Jang et al., 2019).

P301S Transgenic Mouse Model

Tauopathies seen in Alzheimer's disease can be studied using the P301S transgenic mouse model. A tau protein variant with the P301S mutation is expressed by mice in this model. The prion protein promoter drives the expression of the mutant tau gene in P301S mice (Lewis et al., 2000).

Hyperphosphorylated tau protein primarily builds up in high-function brain areas including the cortex and hippocampus in these animals, impairing cognitive and motor abilities. Behavioral tests including the Morris water maze and orientation tasks have shown decreased learning and memory ability in P301S mice (Higuchi et al., 2002). Muscle atrophy and coordination issues, which show up as irregular gait, poor postural stability, and slow walking speed, are signs of motor impairment (Creed et al., 2024).

From two to three months of age, P301S transgenic mice have been shown to exhibit behavioral abnormalities such as

increased activity, inappropriate weight bearing, and defective nest-building. Usually, the third month is when motor impairment first appears. Between 3 and 4 months of age, motor impairments become more noticeable, and they advance quickly until about 5.5 months of age. Tau buildup in the cerebellar nuclei coincides with this process. The P301S model is appropriate for tracking the development and course of the disease, according to these early behavioral and motor deficits (Yin et al., 2016).

P301S mice show significant hippocampus atrophy and neuronal death by the time they are 9–12 months old (Longo et al., 2024). Hyperphosphorylated tau buildup has also been found in retinal ganglion cells in this experimental Alzheimer's model, which is similar to the retinal degeneration seen in Alzheimer's disease (Gasparini et al., 2006). Strategies to reduce tau buildup have often been tested and evaluated using the P301S model. For instance, it has been demonstrated that quercetagetin therapy improves cognitive performance while reducing tau aggregation and deposition (Zhong et al., 2023). Another study showed a decrease in tau buildup and related behavioral deficits utilizing a molecular tweezer called CLR01 (Di et al., 2021).

rTg4510 Transgenic Mouse Model

The human tau protein with the P301L mutation is expressed in the rTg4510 transgenic mouse strain. Two different mouse lines are crossed to create this model. One line expresses the tetracycline transactivator (tTA) gene under the CaMKIIα promoter, while the other contains the mutant human tau gene under the control of a tetracycline operator (tetO) (SantaCruz et al., 2005). Due to this double-transgenic system, mutant tau is mostly expressed in forebrain regions, specifically the cortex and hippocampus, and doxycycline can lower its level (Ramsden et al., 2005).

Pathologies linked to tau overproduction appear early in this transgenic mouse model. Mature neurofibrillary tangles (NFTs) start to form at 4 months of age, whereas tau hyperphosphorylation occurs around 2-3 months of age (Hoover et al., 2010). Both cortical and hippocampal regions have shown significant neuronal loss by 5–6 months of age (Spires et al., 2006).

In this scenario, pathogenic alterations in the brain develop at the same time as cognitive impairment. Mice perform worse on cognitive tests like the Morris water maze and object recognition tests starting at 4 months of age, when NFT formation becomes apparent. According to Ramsden et al. (2005) and SantaCruz et al. (2005), poor performance on these tasks is thought to be a significant predictor of deficits in spatial memory and learning skills. Another study by Yue et al. (2011) showed that this model shows decreased motor activity and anxiety-like behaviors in addition to cognitive deterioration.

This transgenic system has a significant benefit over previous models in that tau expression can be controlled using doxycycline, which is especially useful for time-dependent research (SantaCruz et al., 2005). As a result, the rTg4510 transgenic mouse model has been extensively employed to study the molecular causes of tauopathies and assess the effectiveness of pharmacological treatments meant to manage tau disease. For example, a study by Spires et al. (2006) offered a thorough examination of this model's progressive dendritic architectural degradation. In a similar vein, another work by SantaCruz et al. (2005) demonstrated that in these animals, tau expression suppression improved cognitive function and decreased neuropathological load.

K3 Transgenic Mouse Model (K369I)

To express the human tau protein with the K369I mutation under the control of the prion protein (Prnp) promoter, the K3

transgenic mouse model was created. Human frontotemporal dementia (FTDP-17) has been linked to the K369I mutation, which is known to interfere with microtubule stabilization and promote protein accumulation and aggregation. According to this hypothesis, early onset of disease-related symptoms is caused by increased production of mutant tau protein in the central nervous system (Ishihara et al., 2001).

Tau hyperphosphorylation, aberrant neuronal morphology, and tau protein accumulation are seen in K3 transgenic mice as early as two months of life. In motor control areas including the brainstem, spinal cord, and cerebellum, these diseased characteristics are more noticeable. As a result, this transgenic model is particularly useful for researching tauopathies that are marked by severe motor impairment (Terwel et al., 2005). Postural instability, decreased spontaneous movement, tremor, and ataxia are among the clinical symptoms that start to show up from 3–4 months of age (Ishihara et al., 2001). Furthermore, myelin degradation and axonal transport deficiencies were noted by Terwel et al. (2005), suggesting that neurodegenerative processes are still present in K3 animals.

The early onset of motor symptoms sets the K3 transgenic model apart from previous tau models. It is thought to be a better model for primary movement disorder-associated tauopathies like progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) than Alzheimer's disease because of the early involvement of the motor system; however, it can still be used as an experimental model for tau pathology related to Alzheimer's disease (Ishihara et al., 2001; Terwel et al., 2005).

The K3 model has been extensively utilized to study the mechanisms driving synaptic plasticity loss and neuroinflammation, as well as motor impairment resulting from tau disease. For instance,

Terwel et al. (2005) showed that tau accumulation in this model correlates with glial responses to injury to the central nervous system, with greater neurodegeneration shown as the disease advances.

Non-transgenic Alzheimer's Disease Models

The term "non-transgenic Alzheimer's disease models" refers to models in which the organism is not genetically altered; instead, pathologies associated with Alzheimer's disease are induced through a variety of methods, including the use of exogenous pharmaceutical agents or surgical procedures (Lecanu et al., 2013).

Pharmacological Models

Pharmacological Alzheimer's disease models use pharmacological agents without genetic mutation to mimic cognitive impairments and neurological changes associated with Alzheimer's disease. These models are typically used to quickly assess the effects of medicinal substances. They might not, however, accurately summarize the long-term pathological characteristics of Alzheimer's disease (Gilles & Ertle, 2000). Pharmacologically produced models are especially useful for researching distinct molecular mechanisms involved in the evolution of the disease as well as for examining disease-related pathologies and symptoms seen in the early stages of Alzheimer's disease (Kamat et al., 2016).

Scopolamine Model

By obstructing the receptors of acetylcholine, a neurotransmitter essential to neural signaling, scopolamine damages the cholinergic system. Scopolamine administration mimics the cholinergic deficiencies seen in Alzheimer's disease by causing significant impairments in learning and memory skills (Ebert & Kirch, 1998).

Intraperitoneal injections of scopolamine, either as a single dosage or in multiple doses, are used to establish these experimental animal models. Behavioral tests like the Morris water maze are commonly used to measure the consequent cognitive function impairments that qualify the model as an experimental portrayal of Alzheimer's disease (Dinel et al., 2011). In a research by Carli et al. (2000), rats treated with scopolamine showed raised levels of cytokines linked to inflammation, increased oxidative stress indicators, and decreased levels of acetylcholine in the hippocampus. In a different investigation, scopolamine treatment resulted in hippocampus inflammation and cognitive impairments (Dinel et al., 2011). Nevertheless, this model does not adequately represent all clinical features of Alzheimer's disease and mainly simulates failure of the cholinergic system.

Dinel et al. (2011) used the Morris water maze, where animals must find a concealed platform in a pool of water, to assess learning and memory abilities in scopolamine-treated animals. They saw a lower success rate in platform acquisition and an extended latency to locate the platform. These results showed that scopolamine significantly impairs cognitive performance.

In a another study that used the raised open-field test to measure emotional states like anxiety and depression, scopolamine-treated rats showed more anxiety-like behavior by spending less time in the open area. This study demonstrated that scopolamine treatment impacts emotional states in addition to cognitive functions (Ebert & Kirch, 1998). Additionally, in the passive avoidance test, which assesses fear learning and memory, scopolamine-treated mice showed a greater failure rate in avoiding electric shock. This finding further demonstrated that scopolamine impairs mice's cognitive capacities related to learning and memory (Ebert & Kirch, 1998).

Streptozotocin (STZ) Model

When streptozotocin is given intracerebroventricularly (ICV), it interferes with insulin signaling in the brain, which stops insulin from carrying out its function in neuronal transmission. The brain's neuronal transmission depends on insulin, and when its signaling is compromised, pathologies similar to those seen in Alzheimer's disease arise. Streptozotocin injection is a reliable animal model of Alzheimer's disease because it causes oxidative stress, neuroinflammation, tau protein hyperphosphorylation, amyloid-β buildup, and other hallmark clinical hallmarks of the disease (Grünblatt et al., 2007). De la Monte and Wands (2008) have shown that in addition to these pathological alterations, synaptic loss has also been documented in the streptozotocin model.

Grünblatt et al. (2007) used histopathological techniques to visually establish the presence of tau hyperphosphorylation and amyloid accumulation in the brain. Additionally, streptozotocin-treated mice showed longer escape latencies and worse success rates in finding the concealed platform in the Morris water maze test used in the same study, suggesting serious deficits in learning and memory abilities.

Aluminum Toxicity Model

It has been demonstrated that prolonged oral or intraperitoneal exposure to aluminum salts, such as aluminum chloride ($AlCl_3$), can cause neurodegenerative alterations (Kumar & Gill, 2014; Exley, 2014). Exposure to aluminum causes a number of pathological processes, including as the buildup of amyloid-β, the hyperphosphorylation of tau proteins, an increase in oxidative stress, and the activation of glial cells, all of which are important in neurodegeneration. Histopathological examinations in a research by Bondy (2010) showed indications of oxidative damage after aluminum administration, neuronal loss, gliosis from damage-

induced glial growth, and the development of amyloid-like deposits in the brain.

The open-field test, which measures characteristics including locomotor activity and exploratory behavior, was used in research by Kumar and Gill (2009) to assess mice exposed to aluminum. Aluminum chloride ($AlCl_3$)-treated mice showed lower exploratory behavior and locomotor activity, suggesting deficits in both motor and cognitive functioning. Results from the Morris water maze test, which revealed that mice exposed to aluminum had longer escape latencies and lower success rates in identifying the concealed platform, provided more proof of cognitive impairment and corroborated these findings (Kumar & Gill, 2009).

D-Galactose Model

High doses of D-galactose administered over an extended period of time cause inflammation and oxidative stress in the mouse brain. D-galactose has been utilized to create an experimental model associated with Alzheimer's disease because these settings resemble pathogenic processes seen throughout aging and Alzheimer's disease. However, this model is better described as an aging model rather than a real Alzheimer's disease model because it mostly replicates aging-related alterations rather than the entire range of Alzheimer's disease pathology (Wei et al., 2005).

Wei et al. (2005) used behavioral tests such the Morris water maze and object recognition tests to assess short-term memory, spatial learning, and recognition of new objects. Using these paradigms, they found that mice given D-galactose showed higher oxidative stress and cognitive deficits.

Lipopolysaccharide (LPS) Model

For the purpose of creating experimental models to comprehend the neuroinflammatory aspect of Alzheimer's disease,

lipopolysaccharides—which are generated from the bacterial cell wall and have harmful effects on the central nervous system—are ideal. Usually injected intraperitoneally or intracerebrally, LPS causes neuroinflammation, microglial activation, and the release of pro-inflammatory cytokines in the central nervous system (Lee et al., 2008).

LPS-treated mice showed longer latency to reach the concealed platform and worse success rates in finding it in the Morris water maze test, which Lee et al. (2008) utilized to evaluate cognitive function. Other cognitive tests including memory, recognition, short-term memory, and reactions to environmental changes also showed deficits. These results show that LPS poisoning causes neuroinflammation-mediated cognitive impairments resembling Alzheimer's.

Aging-Induced Models

One of the biggest risk factors for Alzheimer's disease is getting older. Aging-based models are widely employed in Alzheimer's disease research because aging animals display pathogenic symptoms seen in Alzheimer's disease, such as tau protein hyperphosphorylation, amyloid-β buildup, and neuroinflammation (Bennett et al., 2006).

Behavioral tests including the Morris water maze, Y-maze, and novel object recognition test are frequently used to evaluate cognitive processes impacted by these pathological alterations. Age-related cognitive decline is demonstrated by older animals' lower learning and memory test success rates and higher task performance latencies as compared to younger animals (Gallagher & Rapp, 1997).

Diet-Induced Models

High-fat diets (HFDs) accelerate the start and progression of pathology associated with Alzheimer's disease by causing insulin

resistance, metabolic syndrome, and obesity. There have been reports of increased oxidative stress, tau protein hyperphosphorylation, amyloid-β buildup, and cognitive function impairments in animal models fed a high-fat diet (Freeman et al., 2014).

Kothari et al. (2017) used behavioral tests like the Morris water maze and the passive avoidance test to assess cognitive abilities in animals fed a high-fat diet. When compared to animals not exposed to a high-fat diet, animals kept on a high-fat diet showed a marked reduction in cognitive abilities, such as learning and spatial memory.

Conclusion

As the world's population ages, Alzheimer's disease has become more common. It is acknowledged as a neurological condition with a complicated etiology. A deeper comprehension of the disease's fundamental causes is crucial to advancing research on its diagnosis and therapy. In this regard, experimental models of Alzheimer's disease are essential resources for understanding the pathophysiology of the condition and evaluating possible treatment approaches.

Both transgenic and non-transgenic models, each of which represents a distinct facet of Alzheimer's disease, are examined within the parameters of this thesis. While non-transgenic models enable the investigation of pharmacological, aging-related, and environmental factors contributing to illness development, transgenic models are especially useful for understanding the consequences of genetic alterations and for studying tau and amyloid-β pathologies.

Cognitive functions can be thoroughly assessed by behavioral tests created with these models, and scientific evidence

can be used to show how the condition progresses and how therapeutic approaches work.

However, no single model can accurately capture the whole clinical process because Alzheimer's disease is complex. As a result, choosing a model should be closely related to the study's goals, and when needed, several models should be utilized in tandem.

In conclusion, experimental models of Alzheimer's disease provide for a deeper comprehension of the condition at the molecular, cellular, and behavioral levels, providing hope for the future development of successful treatment approaches. Researchers can choose the best experimental models using the material in this thesis as a framework.

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ROLE OF PYROPTOSIS IN CANCER

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Introduction

For many years, cancer has been based on the idea of uncontrolled cellular proliferation, but the most significant ability of cancer cells is their ability to evade programmed cell death. For decades, apoptosis has represented the most common model of programmed cell death. Apoptosis is an orderly cellular disintegration process that does not disrupt the surrounding tissue architecture and does not provoke inflammation (Carneiro & El-Deiry, 2020). The main aim of many classic cancer therapies, including chemotherapy and radiotherapy, has been to restore the apoptotic mechanism, which malignant cells skillfully turn off. However, the frequent development of resistance to apoptosis led to finding alternative mechanisms to induce the death of cancer cells.

The continued discovery and characterization of novel cell death forms have opened new horizons in cancer biology and therapy. Among these, one of the most interesting is pyroptosis, a name derived from the Greek words pyro, meaning fire, and ptosis,

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meaning falling. First described in the 1990s in macrophages infected with Shigella flexneri or Salmonella, pyroptosis was initially confused with apoptosis because it is associated with DNA fragmentation and caspases (Zychlinsky et al., 1992; Hilbi et al., 1998). However, its distinctive, highly inflammatory response was described at the beginning of the 2000s, after which it was classified as a distinctive form of cell death (Cookson & Brennan, 2001).

Unlike apoptosis, which does not induce an inflammatory response, pyroptosis is a form of programmed necrotic cell death that is characterized by the rupture of the cell membrane, osmotic lysis, and the release of pro-inflammatory cytokines and damage-associated molecular patterns. This cellular explosion acts as a potent alarm system to alert the immune system to the presence of pathogens or cellular damage (Bergsbaken et al., 2009). For many years, research into pyroptosis was limited to the fields of immunology and infectious diseases. In recent years, however, especially with a series of findings including identification of the gasdermin family involved in the pyroptosis process, pyroptosis has become increasingly important in cancer research as well (Shi et al., 2015; Kayagaki et al., 2015).

Gasdermins and Pyroptosis Mechanisms

Among the pyroptosis mechanisms that have been identified, a family of pore-forming proteins referred to as gasdermins is the key mediator. So far, six members include GSDMA, GSDMB, GSDMC, GSDMD, GSDME, also known as DFNA5, and DFNB59 (Tam et al., 2021). Proteins belonging to this family share a conserved two-domain structure that consists of an N-terminal domain, known as GSDM-NT, possessing intrinsic cytotoxic pore-forming capability and a C-terminal domain, referred to as GSDM-CT, representing an auto-inhibitory repressor. In resting cells,

GSDM-CT interacts with GSDM-NT and silences it from causing spontaneous membrane rupture and cell death (Liu et al., 2016).

Pyroptosis is induced by the critical proteolytic cleavage of a gasdermin protein at a specific linker region, consequently releasing the active GSDM-NT fragment from its inhibitory counterpart. Once released, GSDM-NT oligomerizes to insert into the inner leaflet of the plasma membrane, forming pores. These pores allow for uncontrolled influx of water and ions, disrupting cellular ion homeostasis. This causes cell swelling, osmotic lysis, and ultimately rupture of the cell membrane (Ding et al., 2016). At this final stage, there is an uncontrolled release of pro-inflammatory intracellular molecules, which has been characterized as a hallmark of pyroptosis.

Two well-defined pathways of cleavage and activation of gasdermins exist: the canonical and non-canonical pathways. The caspases in both the pathways are then activated. More recently, caspase-3-mediated cleavage of GSDME has been identified as an important mechanism linking traditional apoptotic stimuli to pyroptotic outcomes in cancer cells.

Canonical Pathway

The canonical pathway is triggered by the cytosolic recognition of pathogen-associated molecular patterns (PAMPs) or host-derived damage-associated molecular patterns (DAMPs) via a set of pattern recognition receptors like NLRP3, AIM2, NLRC4, or Pyrin. These receptors, upon binding their ligands, oligomerize and build a multiprotein complex called the inflammasome. The inflammasome subsequently promotes the activation of the inflammatory caspase, pro-caspase-1, through the adaptor protein ASC. The active caspase-1 further executes two critical tasks: (i) maturation and release of the proinflammatory cytokines IL-1β and IL-18; and (ii) cleavage of GSDMD at an aspartate residue,

liberating the active GSDMD-NT fragment and executing pyroptosis (He et al., 2015; Kayagaki et al., 2015).

Non-Canonical Pathway

This pathway is specifically activated by intracellular bacterial lipopolysaccharide of Gram-negative bacteria. The cytosolic LPS is directly recognized by caspase-4 and -5 (caspase-11 in mice), which undergo autoproteolytic activation. Unlike the canonical pathway, these caspases do not require an inflammasome complex for their activation. Once active, caspase-4/5/11 directly cleave GSDMD to trigger pyroptosis in a caspase-1-independent manner (Shi et al., 2014; Kayagaki et al., 2011).

Caspase-3/GSDME Pathway: A Bridge from Apoptosis to Pyroptosis in Cancer

A major discovery in cancer biology was the fact that caspase-3, the key executioner caspase in the initiation of apoptosis, is able to cleave and activate gasdermin E. Using GSDME-expressing cells. Wang et al., 2017 showed that the activation of caspase-3 by classic apoptotic stimuli leads to the cleavage of GSDME. Cleavage of GSDME turned the cell death mechanism from apoptosis to highly inflammatory pyroptosis. These findings demonstrated a link between classic cancer therapies and pyroptosis.

GSDME expression level is a key determinant of the death fate of a cell. Numerous malignant cells epigenetically silence the GSDME gene via promoter hypermethylation (Kim et al., 2008). By contrast, cancer cells or normal cells with residual expression of GSDME are prepared to undergo pyroptosis upon activation of caspase-3. Moreover, other granzyme proteases, such as Granzyme B, from cytotoxic lymphocytes can also cleave and activate specific gasdermins, for instance, GSDMB and GSDME, respectively,

reflecting another pathway leading to the initiation of pyroptosis (Zhou et al., 2020; Zhang et al., 2020)

Anti-Tumor Role of Pyroptosis

Stimulating the pyroptosis process in tumor cells can turn a tumor marked by absence of T-cell infiltration and an immunosuppressive microenvironment into a tumor replete with activated immune cells. This transformative effect is almost completely accompanied by gasdermin-mediated pore formation and plasma membrane rupture. The lytic release of cellular contents during pyroptosis converts the tumor microenvironment into an immunostimulatory signal-rich environment.

Pro-inflammatory Cytokines

As part of pyroptosis, the inflammasome-caspase-1 axis immediately results in the production of mature forms of IL-1β and IL-18. To this end, IL-18 plays an important role in stimulating interferon-gamma (IFN-γ) secretion from NK cells and T cells that drives augmented cytotoxic activities against tumor cells (Vidal-Vanaclocha et al., 2000). While IL-1β represents a complex molecule involved in many processes, an acute burst of this cytokine may be responsible for immune activation.

Damage-Associated Molecular Patterns (DAMPs)

The classic DAMPs released by pyroptotic membrane rupture include ATP, high mobility group box 1, heat shock proteins, and DNA. ATP is a potent chemoattractant for DCs and other myeloid cells through the P2X7 purinergic receptor (Ghiringhelli et al., 2009). HMGB1, after binding to its receptors, induces DC maturation and antigen presentation (Apetoh et al., 2007).

Tumor-Associated Antigens

Pyroptotic membrane rupture causes abundant releases of tumor neoantigens and other intracellular proteins, providing a source for T cell activation.

Wang et al. (2017) demonstrated the critical role of GSDME in chemotherapy-induced anti-tumor effects. The study observed that GSDME-expressing breast cancer and melanoma cells underwent pyroptosis in response to chemotherapeutic agents like cisplatin and doxorubicin, leading to HMGB1 release and a significant increase in tumor-infiltrating lymphocytes, including CD8+ T cells and NK cells, which strongly suppressed tumor growth in mouse models. This effect was completely abolished in GSDME-deficient mice, confirming that this immunogenic effect was specifically dependent on pyroptosis.

Beyond GSDME, approaches targeting other gasdermins are also being pursued. Nanoparticle-mediated or engineered bacterial system delivery of the active GSDMD-NT or GSDME-NT fragments into tumor cells has been able to directly induce pyroptosis, elicit a potent anti-tumor immune response, and even eradicate established tumors while establishing long-term immunological memory in preclinical models (Huang et al., 2025; Zhou et al., 2020).

Pro-Tumorigenic Role of Pyroptosis

Despite such potent anti-tumor activity, the intrinsically inflammatory nature of pyroptosis also has grave pro-tumorigenic effects. Chronic inflammation is one of the well-known cancer-promoting processes, playing a critical role in every type of cancer development (Greten & Grivennikov, 2019). Inflammatory mediators, which may be useful for the stimulation of immune defense in an acute and controlled context, by constantly being

present, may establish a microenvironment that fuels almost every hallmark of cancer, including proliferation, angiogenesis, invasion, and metastasis. These aspects of tumorigenesis have been mainly attributed to the continuous release of cytokine products, such as IL-1β, and the recruitment of immunosuppressive cells associated with pyroptosis.

Chronic Inflammation

Sustained activation of the NLRP3 inflammasome and subsequent IL-1β production has been strongly implicated in driving tumorigenesis in various models. This is best documented in studies of colitis-associated cancer (CAC), a classic model of inflammation-driven cancer. These have demonstrated that chronic damage in the colonic epithelium leads to persistent DAMP release, NLRP3 inflammasome activation, and pyroptosis in intestinal epithelial cells. Resulting chronic elevation of IL-1β and IL-18 can activate NF-κB and other signaling pathways in both epithelial and stromal cells to influence the expression of proliferative cytokines such as IL-6 and angiogenic factors including VEGF. Cellular damage associated with chronic inflammation ultimately emerges as a tumor-promoting factor. In these models, inhibition of IL-1β signaling is thought to significantly reduce tumor burden (Allen et al., 2010; Zaki et al., 2010).

Immunosuppression

Inflammatory cytokines released during pyroptosis can also activate immune-suppressing cells. IL-1β is a strong activator of myeloid-derived suppressor cells (MDSCs). MDSCs represent a heterogeneous population of immature myeloid cells that possess a strong immunosuppressive activity through the production of arginase, reactive oxygen species, and other suppressive factors against CD8+ T cell and NK cell activities. Additionally, the resultant inflammatory environment favors the differentiation of

regulatory T cells (Tregs), which further dampen the anti-tumor response. Thus, in progressive tumors, pyroptosis in a minority of cells forms a shield that protects the majority from immune attack (Carneiro et al., 2007).

Metastasis

Pyroptosis may also play a protumorigenic role in promoting cancer metastasis. Such a process concerning the invasive properties of tumor cells involves the remodeling of the ECM. In the inflammatory environment caused by pyroptosis, one can find a high level of MMPs, which are enhanced by cytokines such as IL-1β, degrading the ECM and breaking physical barriers that might create conduits for cancer cell dissemination (Wu et al., 2021). Besides, it is supposed that the hallmark features of pyroptosis facilitate the entry of viable, aggressive tumor cell clusters into the circulation or lymphatic system.

The Dual Role of Pyroptosis

The dual role of pyroptosis in cancer is attributed to many aspects of the tumor microenvironment. Elucidating these variables will be essential for predicting the outcomes of therapeutic interventions aimed at modulating pyroptosis. Gasdermin expression profiles are highly significant. For instance, the expression of GSDME in cancer cells can predispose them to chemotherapy-induced pyroptosis and immunogenic cell death. In contrast, the expression of other gasdermins, such as GSDMC, offers a switch point towards a transition from apoptosis to pyroptosis driven by TNF-α and caspase-8 in macrophages, which may contribute to a pro-tumorigenic environment (Hou et al., 2020). Moreover, the induction of pyroptosis may trigger an immune response against existing immunosuppression in the tumor microenvironment, while in cases of chronic pyroptosis, it may gradually create a pro-tumorigenic inflammatory environment.

The cell type undergoing pyroptosis is extremely important. Pyroptosis in cancer cells, in general, is desirable because of its direct cytotoxic effects and due to its immunogenic effect. However, pyroptosis in critical anti-tumor immune cells, such as T cells, can be greatly harmful and lead to immunosuppression. Certain pathogens or tumor-derived factors can induce pyroptosis in T cells as an immune evasion strategy (Wu et al., 2021).

Conclusion

Pyroptosis has rapidly evolved from a specialized mechanism of host defense against pathogens into a central player in the complex landscape of cancer. Its role is inherently dualistic, representing the balance of inflammation in oncology. It represents one of the most powerful mechanisms of immunogenic cell death, with the capacity to activate immune responses that can eradicate tumors and establish long-term immunity. On the other hand, when dysregulated or chronic, it mediates tumor growth, immunosuppression, and metastasis.

Unraveling this complex process holds the key to the therapeutic potential of pyroptosis. Pyroptosis is not a pathway that can be simply "turned on" or "turned off." On the contrary, the research agenda over the next decade involves revealing the factors underlying the outcome of pyroptosis and developing the corresponding therapeutic tools that can manipulate it with precision for patient benefit. This calls for an in-depth study of the biology of individual gasdermins, tumor microenvironment dynamics, and the intersection of pyroptosis with other cell death and immune signaling pathways. As we continue to unravel the processes controlling this "fiery" form of cell death, strategic induction of pyroptosis bears the potential to target tumors in a controlled and effective manner, poised to become a cornerstone of next-generation cancer immunotherapy.

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THE ROLE OF BIOMARKERS IN VETERINARY MEDICINE FOR DIAGNOSIS, PROGNOSIS, AND TREATMENT MONITORING

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Introduction

Biomarkers are powerful diagnostic tools that objectively measure and evaluate normal biological processes, pathological conditions or response to treatment in animals. This study aims to comprehensively review the critical role of biomarkers in veterinary medicine, their clinical application areas (diagnosis, prognosis, treatment monitoring), interspecies differences in use and current limitations

Evolving from traditional blood biochemistry parameters to more specific markers based on proteomics and genomics, this field has increased the effectiveness of veterinary medicine in a wide range of areas, from individual patient treatment to herd health

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management and public health. However, the lack of standard reference intervals, biodiversity and economic factors pose significant limitations. In the future, with the spread of multi-marker panels, artificial intelligence integration and portable diagnostic devices, these challenges are expected to be overcome, and the practice of veterinary medicine is expected to further evolve.

Definition of Biomarkers and Their Significance in Veterinary Medicine

Biomarkers are biological indicators that serve to objectively measure normal biological processes, pathological changes or response to treatment in animals (B. D. W. Group, 2001). As in the NIH and FDA definitions, biomarkers in veterinary medicine are divided into various subgroups including diagnostic, prognostic, predictive, pharmacodynamic and safety markers (B. D. W. Group, 2001; F.-N. B. W. Group, 2016). Thus, they not only detect the presence of disease, but also provide the possibility to predict the course of the disease, choose the appropriate treatment approach and monitor the response to treatment.

Historically, biomarkers used in veterinary medicine were mostly based on blood biochemistry and hematology parameters (urea, creatinine, ALT, AST, hematocrit, etc.) (Myers, Smith, & Turfle, 2017). Today, proteomic, genomic and metabolomic approaches are facilitating the development of much more specific markers for clinical use. For example, Serum Symmetric Dimethylarginine (SDMA) is more sensitive than classical creatinine measurement in the early detection of chronic kidney disease in cats (Hall, Yerramilli, Obare, Yerramilli, & Jewell, 2014; Pereira, Jota Baptista, Faustino-Rocha, Oliveira, & Coelho, 2025; Relford, Robertson, & Clements, 2016) Similarly, acute phase proteins such as Serum Amyloid A (SAA) and C-reactive protein (CRP) are widely used to assess inflammation in dogs and cats

(Ceron, Eckersall, & Martynez-Subiela, 2005; Cocchetto, Zoia, Aragao, Ventura, & Menchetti, 2023; Cray, 2012).

The importance of biomarkers in veterinary medicine is not limited to individual animal health. They also play critical roles in herd and farm management, animal welfare, reproductive performance, and the control of zoonotic diseases. For example, milk amyloid A assays, which detect subclinical forms of mastitis in dairy cows at an early stage, both reduce economic losses and provide advantages in terms of food safety (Gerardi et al., 2009).

Additionally, biomarkers enable veterinarians to indirectly contribute to human health within the One Health approach. Cardiac troponin, NT-proBNP, or specific acute phase proteins used in animals contribute to early diagnosis in domestic animals and support a better understanding of zoonotic and shared pathologies (Oyama & Singletary, 2010).

Portable point-of-care devices and rapid test kits developed in recent years enable the use of biomarkers even in field settings. This allows veterinarians to make rapid diagnosis and treatment decisions even in rural areas, protecting both animal welfare and public health (Manessis, Gelasakis, & Bossis, 2022).

Role in Diagnosis, Prognosis and Treatment Monitoring

Biomarkers provide critical insights at all stages of diagnosis, prognosis, and treatment monitoring in veterinary medicine. In terms of diagnosis, their most significant advantage is that they provide earlier and more specific information compared to traditional laboratory tests. Molecular biomarkers (e.g., PCR-based markers) can detect the presence of disease before clinical symptoms appear by directly identifying the genetic material of pathogens (Eman et al., 2025; Myers et al., 2017). This is particularly important in the

rapid diagnosis of cases such as subclinical mastitis, early renal failure or viral infections(Eman et al., 2025) .

In prognosis prediction, biomarkers provide insights into the course of the disease and its likely progression. For example, evaluating plasma protein profiles in canine mammary tumors can indicate tumor aggressiveness (Park et al., 2020), while elevated cardiac troponin or NT-proBNP levels reflect the severity of heart muscle damage and facilitate predictions regarding survival time (Cray, 2012; Langhorn & Willesen, 2016). This allows clinicians to prioritize cases that require early and intensive treatment (F.-N. B. W. Group, 2016).

From a treatment monitoring perspective, biomarkers provide objective parameters beyond clinical findings. Acute phase proteins (e.g., SAA, CRP) decline shortly after treatment begins, reflecting treatment efficacy (Cray, 2012). Similarly, monitoring glucose and insulin levels in diabetic cats allows for personalized medication dosing. In the long-term follow-up of chronic diseases, markers such as SDMA also contribute to the re-adjustment of treatment protocols by revealing disease progression at an early stage(Hall et al., 2014) .

Biomarkers also play an important role in drug safety and toxicity assessments. In preclinical studies conducted before human clinical trials begin, the lowest toxic dose or highest safe dose is estimated by measuring biomarkers in laboratory animals. For example, the cardiac troponin test used in mice helps identify the risk of cardiac structural damage, thereby contributing to the selection of appropriate dose ranges for human trials (F.-N. B. W. Group, 2016; O'Brien et al., 2006).

Inter-species differences are also an important aspect in the use of biomarkers in veterinary medicine. While CRP is a reliable marker of inflammation in dogs, Serum Amyloid A (SAA) is

considered a more suitable alternative in cats because it does not show the same sensitivity. Similarly, the variances in reference ranges across different races and age groups are an important point to consider in clinical interpretation (Cray, 2012).

The use of biomarkers is not limited to small animal clinics. Other important applications include protecting herd health in large and small animals, early diagnosis of subclinical infections, and monitoring hormone profiles in reproductive management (Cray, 2012; Samardzija et al., 2020). Furthermore, the use of biomarkers in both conservation and rehabilitation programs for exotic and wild animals is also increasing (Cray, 2012).

Looking to the future, multi-biomarker panels promise more reliable results compared to single parameters (F.-N. B. W. Group, 2016). In addition, artificial intelligence-supported decision systems will enable more accurate prognosis predictions by integrating biomarker data with clinical parameters (Ezanno et al., 2021). And lastly, portable point-of-care devices make treatment monitoring easier even in rural and field conditions, which increases the practical applicability of veterinary medicine (Manessis et al., 2022).

Clinical Applications of Biomarkers

Biomarkers have become an indispensable part of many clinical processes in veterinary medicine, such as diagnosis, prognosis, treatment monitoring, and drug safety. Compared to traditional laboratory methods, they provide faster, more sensitive, and often more economical results, making them the preferred choice for both individual patient management and herd health applications (Cray, 2012; Myers et al., 2017).

Diagnosis and early disease detection are among the most common areas where biomarkers are utilized. Serum Symmetric Dimethylarginine (SDMA) can detect chronic kidney disease in cats

earlier than conventional creatinine measurements (Hall et al., 2014); in dairy cows, serum amyloid A levels can indicate the subclinical form of mastitis before clinical signs develop (Cray, 2012). Similarly, NT-proBNP measurement enables the early diagnosis of asymptomatic heart disease in dogs and cats (Fox et al., 2011).

In prognosis assessment, biomarkers provide invaluable information for predicting the course of the disease. Elevated levels of LCAT in plasma proteins in canine mammary tumors indicate that the tumor is more aggressive and survival time is shorter (Park et al., 2020) . Cardiac troponin and NT-proBNP measurements play an important role in prognosis prediction by revealing the degree of heart muscle damage (Langhorn & Willesen, 2016).

In monitoring chronic diseases, biomarkers help objectively assess treatment effectiveness and disease progression(F.-N. B. W. Group, 2016). Regular monitoring of glucose and insulin levels in diabetic cats allows for individualized medication dosing (Sparkes et al., 2015), while SDMA measurements in chronic kidney disease can reveal disease progression earlier than traditional parameters (Hall et al., 2014).

Assessing the response to treatment is one of the most practical functions of biomarkers in clinical practice. Acute phase proteins, particularly Serum Amyloid A (SAA) and C-reactive protein (CRP), reflect the recovery process by declining shortly after treatment begins. With these characteristics, veterinarians can monitor the effectiveness of treatment with numerical data in addition to clinical findings (Cray, 2012).

Drug safety and the assessment of side effects are also areas where biomarkers are used. Before moving on to clinical trials in humans, biomarkers such as cardiac troponin tests in preclinical studies conducted on laboratory animals help determine the lowest

toxic dose and the highest safe dose by revealing any structural damage. This approach both enhances safety in animal experiments and guides the transition to human studies (F.-N. B. W. Group, 2016; O'Brien et al., 2006).

From the perspective of preventive medicine and herd management, biomarkers offer substantial contributions in screening for subclinical infections and in reproductive management (Cray, 2012). The quantification of biomarkers such as progesterone and anti-Müllerian hormone (AMH) facilitates fertility management in dogs, cattle, and horses, enhancing economic efficiency by predicting the optimal time for insemination (Mossa et al., 2015).

Importantly, biomarkers also hold value within the context of public health and the One Health perspective. The early diagnosis of zoonotic diseases, the tracking of epidemiological data, and the assurance of food safety can be performed more effectively (Cray, 2012; F.-N. B. W. Group, 2016). In the future, the role of biomarkers in clinical applications will be further strengthened with the proliferation of multi-biomarker panels (F.-N. B. W. Group, 2016), artificial intelligence-based analyses (Ezanno et al., 2021), and portable point-of-care devices (Manessis et al., 2022).

Commonly Used Biomarkers in Clinical Practice

The clinical use of biomarkers in veterinary medicine is quite wide; however, in daily practice, some markers are much more frequently preferred than others for diagnosis, prognosis, and treatment monitoring. This section examines the most commonly used biomarker groups and their clinical applications (Cray, 2012).

Hematological and biochemical markers are the cornerstones of veterinary clinics. In liver diseases, ALT, AST, ALP, and GGT levels are frequently used to assess hepatic function. Urea and creatinine are commonly used parameters for monitoring kidney

function, and SDMA is now also widely used in addition to these (Hall et al., 2014). Glucose and insulin levels are critical for the diagnosis and monitoring of diabetes. In addition, complete blood count (RBC, WBC, hemoglobin, hematocrit) is one of the most commonly used hematological biomarkers in veterinary medicine for routine screening and infection assessment (Myers et al., 2017).

Inflammatory markers are prominent in the diagnosis and monitoring of infections and inflammatory processes. Serum Amyloid A (SAA) and C-reactive protein (CRP) are the most commonly recognized acute phase proteins. While CRP is a reliable marker in dogs, SAA is more widely used in cats due to CRP's limited sensitivity. Additionally, haptoglobin and fibrinogen are biomarkers used to assess inflammation, particularly in large animal practice (Cray, 2012).

Endocrine biomarkers play a critical role in assessing hormonal imbalances. T4, T3, and TSH are used in the diagnosis of thyroid dysfunction (hypothyroidism, hyperthyroidism), while cortisol and ACTH measurements are important in the differential diagnosis of Addison's and Cushing's diseases(Behrend, Kooistra, Nelson, Reusch, & Scott-Moncrieff, 2013; Scott-Moncrieff, 2012). In reproductive management, progesterone and AMH (Anti-Müllerian Hormone) are the most commonly used biomarkers for fertility monitoring(Mossa et al., 2015).

Oncological biomarkers are becoming increasingly recognizable in veterinary oncology. Immune markers such as CD3, CD79a, and Pax5 are used in the diagnosis and classification of lymphomas (Valli et al., 2011); additionally, specific tumor antigens and biomarkers targeting genetic mutations guide clinicians in the early diagnosis of different cancer types and in determining prognosis.

Cardiac biomarkers are also commonly used parameters in small animal practice. Troponin I and Troponin T are used as specific indicators of heart muscle injury (Langhorn & Willesen, 2016). In addition, NT-proBNP provides valuable information to clinicians in the diagnosis of heart failure, particularly in dogs and cats, and in the early detection of subclinical cardiac disorders (Fox et al., 2011).

As a result, the most commonly used biomarkers in clinical practice are grouped into hematological, biochemical, inflammatory, endocrine, oncological, and cardiac categories. These markers provide veterinarians with speed and accuracy in the diagnostic process, while also offering reliable information for prognosis and treatment monitoring (Cray, 2012; Myers et al., 2017).

Table 1. Common Biomarkers and Their Application in Veterinary Medicine

Marker Group	Sample	Area of Application	Clinical Note
Hematological & Biochemical	ALT, AST, ALP, GGT; Urea, Creatinine, SDMA; Glucose	Liver and kidney function; diabetes, routine hematology	SDMA elevates before creatinine; complete blood count is fundamental in screening
Inflammatory	SAA, CRP, Haptoglobin, Fibrinogen	Early diagnosis and monitoring of infection and inflammation	CRP is reliable in dogs; SAA is preferred in cats
Endocrine	T4, T3, TSH; Cortisol, ACTH; Progesterone, AMH	Thyroid disorders; Addison's & Cushing's; fertility monitoring	In reproductive management, progesterone and AMH increase herd productivity
Oncological	CD3, CD79a, Pax5; Tumor antigens	Lymphoma classification; tumor diagnosis and prognosis determination	It must be confirmed by immunohistochemistry.
Cardiac	Troponin I, Troponin T, NT-proBNP	Heart muscle injury; heart failure, subclinical cardiac diseases	NT-proBNP detects latent heart disease.

Biomarkers Used in Human Medicine but with Limited Use in Veterinary Medicine and Those Used in Both Fields

Although biomarkers play an important role in diagnosis, prognosis, and treatment monitoring in both human and animal

health, not all biomarkers are equally valid or applicable. While some biomarkers are widely used in human medicine, they have limited value in veterinary medicine or are unsuitable due to interspecies differences (Cray, 2012). However, some indicators provide reliable information in both areas and thus find common ground (Eckersall & Bell, 2010).

Among biomarkers that are rarely used or unsuitable in veterinary medicine, those that are standard in human medicine but have limited diagnostic value in animals are particularly noteworthy. For example, C-reactive protein (CRP) is the most commonly used inflammation marker in humans, but it is unreliable in cats due to its low sensitivity (Cray, 2012).

Similarly, while prostate-specific antigen (PSA) plays a critical role in the early diagnosis of prostate cancer in men, it is not suitable for diagnostic use in domestic animals(Bell, Klausner, Hayden, Feeney, & Johnston, 1995).Furthermore, tumor markers commonly used in human medicine (such as CA 125 and CA 19-9) have not been validated in animals and do not have a meaningful place in veterinary clinical practice (Colombe, Beguin, Benchekroun, & Le Roux, 2022).

In contrast, commonly used biomarkers carry high diagnostic and prognostic value in both fields. Cardiac troponin I/T and NT-proBNP are reliable parameters for assessing cardiac damage and heart failure in both humans and dogs and cats(Fox et al., 2011; Langhorn & Willesen, 2016). SDMA, used in the follow-up of kidney diseases, stands out in both human and veterinary medicine for its ability to indicate early renal dysfunction (Hall et al., 2014). In addition, acute phase proteins such as Serum Amyloid A (SAA) and haptoglobin are valuable biomarkers for monitoring inflammatory processes in both humans and animals, despite some species differences (Cray, 2012).

In conclusion, there are both similarities and differences between the uses of biomarkers in human and veterinary medicine. This situation highlights the importance of interspecies biological diversity on the one hand, while on the other hand demonstrating that commonly used markers within the One Health approach can guide translational research (Cray, 2012; Eckersall & Bell, 2010).

Table 2. Usage and Limitations of Biomarkers Across Human and Veterinary Medicine

Usage Status	Biomarker	Utility in Human Medicine	Utility in Veterinary Medicine
Limited / Not Suitable	CRP (C-reactive protein)	The most common marker of inflammation	Reliable in dogs; low sensitivity in cats
Not Suitable	PSA (prostate-specific antigen)	The standard in prostate cancer diagnosis	Not applicable to pets
Not Suitable	CA 125, CA 19-9	Markers for gynecological and gastrointestinal cancers	Not verified in veterinary medicine
Jointly Used	Troponin I/T	Diagnosis of myocardial damage	Diagnosis of cardiac injury in dogs and cats
Jointly Used	NT-proBNP	Heart failure and subclinical heart disease	Heart failure and latent heart disease in dogs and cats
Jointly Used	SDMA	An indicator of early kidney dysfunction	Diagnosis of chronic kidney disease in cats and dogs
Jointly Used	SAA, Haptoglobin	Monitoring inflammatory process	It is used in monitoring inflammation in spite of species differences.

Difficulties and Limitations in Use

Although the importance of biomarkers in veterinary medicine is increasing, there are various obstacles in terms of reliability and standardization in practice. These obstacles stem not only from biological diversity but also from methodological, economic, and ethical factors (Cray, 2012).

Variability Among Species and Breeds

- Genetic and proteomic variations: Variations in DNA and proteome levels across different species limit the cross-species use of biomarkers. For example, while the same biomarker antibody may function correctly in a dog, it may be ineffective in a cat due to minor differences in amino acid sequences (Cray, 2012).
- Breed-specific variations: It is known that hematological and biochemical values differ significantly among different breeds of dogs, such as Labradors or Greyhounds. Therefore, the reliability of biomarkers remains limited without establishing breed-based reference ranges(Campora, Freeman, Lewis, Gibson, & Jacobson, 2011).
- Ecological factors: Differences in nutrition, stress, and exposure to environmental toxins between domestic animals, farm animals, and individuals in the wild can significantly alter biomarker levels. For example, oxidative stress markers are much higher in free-roaming dogs than in those living indoors(Passantino, Quartarone, Pediliggeri, Rizzo, & Piccione, 2014) .

Lack of Standard Reference Ranges

- Small population issues: Most veterinary research is conducted on a limited number of animals, so the reference values obtained cannot be generalized (Cray, 2012).

- Age and physiological condition differences: Even within the same species, biomarker levels differ between young, adult, and elderly individuals. For example, acute phase protein baseline levels are lower in young calves than in adults because their immune systems are not fully mature (Cray, 2012).
- Lack of database: While standard ranges are developed in human medicine through international consortia and multicenter studies, such extensive databases have not yet been established in veterinary medicine (Cray, 2012).

Limitations in Clinical Use

- Delayed response: The elevation of certain biomarkers (e.g., serum amyloid A) after clinical symptoms limits early diagnosis (Cray, 2012).
- Low specificity: CRP elevation in both infection and trauma can lead to false positives (Cray, 2012).
- Subclinical diseases: Cardiac Troponin I has limited sensitivity in the subclinical period because it does not rise in the early stages of heart disease (Langhorn & Willesen, 2016).
- Risk of reliance on a single marker: In clinical practice, relying on a single biomarker often increases the risk of misinterpretation. The current trend is toward the use of multiple biomarker panels and “omics”-based profiles .

Economic and Ethical Constraints

- Cost: While most diagnostic tests in human medicine are covered by health insurance, in veterinary medicine, test costs are generally covered by the animal owner. This limits the accessibility of biomarker tests (Eckersall & Bell, 2010).

- Herd health scale: While an expensive test may be feasible for a single animal, the same approach is not economically viable for herds consisting of hundreds of animals (Cray, 2012).
- Ethical factors: Additional invasive procedures for biomarker testing, especially in laboratory animals used in research, may be ethically controversial (Cray, 2012).

Long-Term Solutions

- Shared databases: Extensive data collected from different countries and clinics will enable a better understanding of species and breed differences (Cray, 2012).
- Portable devices: Small devices that enable rapid testing in the field will facilitate early diagnosis (Manessis et al., 2022).
- Artificial intelligence: Complex data from multiple biomarkers can be analyzed using artificial intelligence to provide practical results to the veterinarian (Ezanno et al., 2021).
- One Health approach: Evaluating human and animal data together will be useful, especially for the early detection of infectious diseases (Eckersall & Bell, 2010).

Cost

Unlike human medicine, in veterinary medicine, the costs of diagnosis and treatment are generally covered directly by animal owners rather than by health insurance or public health systems (Eckersall & Bell, 2010). This situation affects the choice of diagnostic tests considered “affordable” in any clinical situation; sometimes inexpensive laboratory tests are preferred, while in some cases empirical treatment or even euthanasia may be considered more economically viable (Eckersall & Bell, 2010). Particularly

when herd health and reproductive performance are at stake, more expensive but faster and more definitive tests may be preferred to protect the health of the entire population or prevent losses during the breeding season(Cray, 2012; Mossa et al., 2015).

In contrast, in human medicine, advanced biomarker tests can be used more readily because diagnostic and treatment costs are covered by public health systems or private health insurance in most countries (Eckersall & Bell, 2010). This financial support facilitates the widespread use of biomarkers in human medicine and the faster introduction of new technologies into clinical practice. In veterinary medicine, however, economic constraints remain one of the most significant limiting factors slowing the transition to routine use of biomarkers (Cray, 2012; Eckersall & Bell, 2010).

Conclusion and Outlook for the Future

Biomarkers are playing an increasingly central role in veterinary medicine in determining diagnosis, prognosis, and treatment strategies (Myers et al., 2017). These tools, which have evolved from classic laboratory parameters to specific molecular-level indicators, contribute to the early and non-invasive diagnosis of diseases, the creation of personalized treatment protocols, and the improvement of animal welfare (Cray, 2012; F.-N. B. W. Group, 2016). Biomarkers such as SDMA, cardiac troponin, NT-proBNP, and SAA, in particular, provide clinicians with valuable information that cannot be obtained through conventional methods, thereby strengthening the clinical decision-making process (Cray, 2012; Fox et al., 2011; Hall et al., 2014; Langhorn & Willesen, 2016).

However, there are still some obstacles to the use of biomarkers. Biological variations between species, races, and individuals; the lack of standardized reference ranges, and economic constraints limit the widespread use of these tests both in individual clinics and in field conditions (Cray, 2012; Eckersall & Bell, 2010).

Among the solutions for the future, the following stand out: establishing species- and breed-specific reference ranges through multi-center studies; integrated analysis of multi-biomarker data using artificial intelligence and machine learning algorithms; and the widespread adoption of point-of-care devices, thereby making rapid, reliable, and affordable testing accessible in rural areas (Cray, 2012; Ezanno et al., 2021; Manessis et al., 2022). Furthermore, strengthening collaboration between human and veterinary medicine within the "One Health" approach will enable translational studies based on common biomarkers and more effective management of zoonotic diseases (Eckersall & Bell, 2010).

As a result, biomarkers have become an integral part of veterinary medicine. With ongoing research and technological innovations, it is anticipated that these valuable tools will be used much more effectively not only in individual animal health but also in herd management, public health, and at the ecosystem level (Cray, 2012; Myers et al., 2017).

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MOLECULAR LANDSCAPES OF BIOCHEMISTRY