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Evidence-Based Medical Therapy for Mitral Regurgitation in Dogs

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Review

Evidence-Based Medical Therapy for Mitral Regurgitation in Dogs

Running Title: Canine MMVD Current and Future Therapy

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Abstract

Myxomatous mitral valve disease (MMVD) is the most common acquired cardiac disease in dogs and a leading cause of congestive heart failure. Over the past three decades, clinical trials and translational studies have generated a substantial evidence base supporting specific medical therapies, particularly drugs that modulate hemodynamics or target the renin–angiotensin–aldosterone system (RAAS). At the same time, gaps remain in our knowledge, and areas of clinical practice are guided more by expert consensus or extrapolation from human medicine than by robust veterinary data. This review summarizes current evidence for pharmacologic interventions in MMVD, including established therapies such as ACE inhibitors, mineralocorticoid receptor antagonists, diuretics, and inodilators, as well as emerging drug classes such as angiotensin receptor–neprilysin inhibitors and SGLT-2 inhibitors. For each, we highlight the existing data, discuss limitations of prior studies, and note areas where additional research is needed to guide optimal clinical use.

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Renin-Angiotensin Aldosterone System Modulation

Biology of the Renin-Angiotensin Aldosterone System in Mitral Regurgitation

Mitral regurgitation (MR) is most commonly the result of MMVD in dogs and leads to a chronic state of volume overload, causing neurohormonal activation. Among these pathways, the RAAS plays a critical role in maladaptive remodeling and fluid retention.

There is limited information on treatment-independent renin-angiotensin alsosterone system (RAAS) activation in dogs with congestive heart failure (CHF). Several clinical trials have demonstrated clear benefits of RAAS modulators, such as angiotensin converting enzyme inhibitors (ACEI) (The COVE Study Group, 1995; The IMPROVE Study Group, 1995; BENCH, 1999; Besche et al., 2007) and mineralocorticoid receptor antagonists (MRA) (Bernay et al., 2010; Coffman et al., 2021), in dogs with cardiac disease. A recent retrospective study by Ward et al. (2021) further reported that higher doses of ACEI were associated with improved survival in dogs at the initial onset of CHF. Within the ACEI-treated canine CHF subgroup, higher doses were associated with greater two-year survival.

Chronic activation of the classical arm of the RAAS (Ang II/aldosterone) results in vasoconstriction, sodium and water retention, sympathetic activation, endothelial dysfunction, and fibrotic remodeling (Packer, 1988; Weber & Brilla, 1991)—providing the rationale for introducing RAAS-modulating strategies, including ACEI and MRA, in the treatment of canine CHF (Ettinger et

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al., 1998; BENCH, 1999). The counter-regulatory (alternative) arm of the RAAS centers on ACE2, which converts angiotensin II (Ang II) to angiotensin(1–7) [Ang(1–7)]. Via the Mas receptor, Ang(1–7) counterbalances AT1 signaling—promoting vasodilation and natriuresis while limiting inflammation, oxidative stress, and fibrosis (Ferrario, 2003; Esteban et al., 2004; Santos, 2018). Accumulating evidence suggests that most RAAS modulators do not only suppress the classical Ang II/aldosterone axis but also activate the counter-regulatory ACE2–Ang(1–7)–Mas pathway (Keidar et al., 2005; Sotillo et al., 2023; Schneider et al., 2023; Masters et al., 2024; Manson et al., 2025).

The RAAS comprises circulating and tissue compartments. In myocardium, kidney, vasculature, and adrenals, ACE-independent pathways (e.g., chymase, cathepsins) generate Ang II, which can drive ACE-inhibitor escape (Weber et al. 1995; Roig et al. 2000). In this context, aldosterone breakthrough—a rebound or rise in aldosterone despite ACEI or angiotensin II receptor blockade (ARB) therapy—has been shown in experimental models and in naturally occurring MMVD (Lantis et al., 2015a,b; Ames et al., 2017; Konta et al., 2018; Mochel et al., 2018, Masters et al., 2024). Proposed drivers of aldosterone breakthrough include incomplete ACE suppression, activation of non-ACE pathways, hyperkalemia-mediated aldosterone stimulation, and increased adrenal sensitivity to Ang II. This feature supports the rationale for combination RAAS therapy rather than monotherapy in the treatment of canine MMVD (Coffman et al., 2021).

Diurnal oscillations in renin–angiotensin–aldosterone activity add a *time* dimension to RAAS regulation, complicating—but also informing—the timing of treatment with RAAS modulators in MMVD. RAAS peptides, blood pressure, and urinary electrolytes fluctuate with a circadian periodicity with a strong influence of feeding schedules in dogs (Mochel et al., 2013b, 2014; Mochel & Danhof, 2015). The timing of dosing and sampling, therefore, can meaningfully alter measured magnitudes of drug effect—an often-overlooked source of between-subject variability in veterinary clinical trials. Such time dependence implies that pharmacodynamic (PD) effects vary across the 24-hour cycle and that suboptimal scheduling can leave "troughs" of incomplete RAAS modulation, particularly in the early morning when renin activity tends to rise (Mochel & Danhof, 2015). Incorporating chronobiology into therapeutic planning therefore represents a possible lever to improve efficacy, following the basic principles of chronotherapy (Tata et al., 2005; Hermida & Ayala, 2009; Martino et al., 2011).

Renin-Angiotensin Aldosterone System Activation, Vascular Inflammation and Remodeling: Lessons from Experimental Models and Human Studies

Excessive activation of the RAAS plays a pivotal role in vascular inflammation and remodeling. The role of chronic inflammation in cardiac diseases was recently emphasized by the American Heart Association (AHA) Presidential Advisory, highlighting the interconnected nature of cardiovascular disease (CVD), chronic kidney disease (CKD), type 2 diabetes (T2DM), and obesity (Ndumele et al., 2023). The Advisory Committee introduced the concept of Cardiovascular–Kidney–Metabolic (CKM) health underscores the interconnected, mutually reinforcing risks of these chronic diseases. They share common pathophysiological pathways—chronic inflammation, oxidative stress, and metabolic dysregulation—that accelerate disease progression and complicate therapeutic management overall (Kadowaki et al., 2022).

Key findings from the literature highlight the contribution of RAAS activation to cardiovascular remodeling:

- **Pro-inflammatory effects of angiotensin II.** Ang II regulates cytokine and chemokine expression in the kidneys, vasculature, and heart, thereby promoting vascular inflammation and remodeling (Matsubara, 1998; Tummala et al., 1999; Pacurari et al., 2014). In experimental models, chronic infusion with Ang II increases blood pressure, induces myocardial infiltration of inflammatory cells, and promotes cardiac fibrosis (Qi et al., 2011).
- **Oxidative stress and end-organ damage.** Ang II-induced oxidative stress and elevated blood pressure contribute to end-organ damage, including myocardial infarction, CHF, and CKD (Devonald & Karet, 2002; Chobanian et al., 2003).

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This body of evidence provides a rationale for targeting the classical RAAS pathway for the treatment of cardiac diseases in both human and veterinary medicine. RAAS modulators, such as ACEI and ARB have been shown to decrease circulating pro-inflammatory markers, including C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), and interleukins such as IL-6 and IL-1β (Fernandez et al., 2008; Krysiak & Okopień, 2011, 2012). They also have an effect on oxidative stress by reducing the production of reactive oxygen species (ROS), thereby preserving endothelial function and preventing vascular damage (Gainer et al., 1998). These anti-inflammatory effects are primarily mediated by blocking Ang II-driven activation of key signaling pathways, notably NF-κB (Li & Zhuo, 2008). Clinically, this translates into lower cardiovascular morbidity and mortality, with particular benefit in patients with chronic inflammatory conditions such as heart failure, hypertension, and kidney disease. Importantly, reductions in inflammatory markers also improve outcomes in patients with metabolic syndrome and diabetes (Vaccari et al., 2008; Bähr et al., 2011).

A systematic review and meta-analysis of 32 randomized controlled trials further supports these findings (Awad et al., 2022). Across 3,489 patients with cardiovascular, metabolic or inflammatory diseases, ACEI significantly reduced inflammatory markers including CRP, IL-6, and TNF-α. Perindopril and ramipril showed marked reductions in IL-6, while enalapril decreased both TNF-α and CRP. ARB reduced IL-6 but showed less consistent effects on other markers, suggesting ACEI may exert stronger anti-inflammatory benefits in cardiovascular disease management.

Beyond ACEI and ARB, MRA such as spironolactone also exhibit pronounced anti-inflammatory and anti-fibrotic effects. The aldosterone–MR axis intersects with canonical inflammatory pathways. TNF-α is a proximal driver of inflammatory cascades via TNFR1 (cell death/inflammation) and TNFR2 (immune modulation/tissue repair), converging on NF-κB–dependent transcription of inflammatory genes (e.g., cytokines, adhesion molecules) (Steele 2023). LPS engagement of TLR4 likewise triggers NF-κB–mediated production of proinflammatory, antiviral, and antibacterial cytokines (Pimentel-Nunes 2010). In individuals at elevated risk of heart failure, spironolactone was associated with greater reductions in biomarkers of collagen turnover, including PICP (Pro-Collagen 1 C-Terminal Pro-Peptide) (Cleland 2021); across heart-failure syndromes, MRA improve cardiac structure and function and are associated with better outcomes (strongest evidence in heart failure (HF) with restricted ejection fraction, emerging in HF with preserved ejection fraction) (Ferreira et al. 2021). Reductions in circulating markers of collagen synthesis have been observed in multiple cardiovascular populations receiving MRA, consistent with antifibrotic activity (Zannad et al., 2000; Pellicori et al., 2020). Mechanistically, in *ex vivo* human blood leukocytes, spironolactone suppresses transcription and secretion of proinflammatory cytokines—including TNF-α, lymphotoxin, IFN-γ, GM-CSF, and IL-6 (Bendtzen 2003); and in macrophage models (RAW 264.7 cells and mouse peritoneal macrophages), it inhibits LPS-induced TNF-α and PGE_2, consistent with attenuation of the IKK/NF-κB pathway (Kato et al., 2014). Taken together, these data support a coherent view in which MR antagonism complements RAAS modulation and engages immunoinflammatory circuits relevant to myocardial and vascular remodeling. Our consortium is actively investigating these immunomodulatory effects of spironolactone and its active metabolites (canrenone, 7-α-thiomethyl-spironolactone) in canine peripheral immune cells and in adult stem cell–derived canine organoid models (Catucci 2025).

Collectively, these earlier reports on ACEI, ARB and MRA underscore the broad anti-inflammatory and anti-fibrotic effects of RAAS modulation and its potential protective role in cardiovascular, renal and metabolic health.

Angiotensin Converting Enzyme Inhibitors: Clinical Efficacy, Pharmacokinetics and Pharmacodynamics

Clinical Efficacy in Congestive Heart Failure (Stage C). Angiotensin converting enzyme inhibitors have been a mainstay of RAAS modulation in MMVD for decades. A series of trials published in the 1990s studied the effects of enalapril vs. placebo added to background management (furosemide +/- digoxin) in dogs with CHF secondary to MMVD or dilated cardiomyopathy (DCM); this review will focus on outcomes from the MMVD cohorts in these studies.

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The first trial, COVE (The COVE Study Group, 1995), followed 141 dogs with MMVD and CHF for 28 days and reported outcomes related to clinical signs and quality of life. Enalapril was initially dosed at 0.5 mg/kg PO q24h, with option to increase dose to q12h based on clinician discretion; approximately 45% of dogs ultimately received twice daily dosing of enalapril. Dogs with MMVD receiving enalapril demonstrated improvement in activity, mobility, total cough score, and overall clinician evaluation compared with placebo at Day 28. Adverse events were equivalent across enalapril and treatment group. The second trial, IMPROVE (The IMPROVE Study Group, 1995), included a separate group of 22 dogs with MMVD and CHF, and reported echocardiographic and invasive hemodynamic measurements after the first dose and after 21 days of dosing of either enalapril or placebo. In this cohort, enalapril was dose consistently at 0.5 mg/kg PO q12h. Results of hemodynamic testing were combined between MMVD and DCM dogs in this study and showed that enalapril-treated dogs had greater decreases in pulmonary capillary wedge pressures, heart rate, and blood pressure compared to placebo in the first 24 hours after dosing. The final trial in this series, LIVE (Ettinger et al., 1998), followed long-term survival outcomes of 67 dogs with MMVD from either the COVE or IMPROVE cohorts. Although the actual median dosage of enalapril was not reported in LIVE, based on dosing regiments for COVE and IMPROVE, dogs received enalapril 0.5 mg/kg PO with approximately half of dogs receiving twice daily dosing. The primary outcome measure in LIVE was treatment failure, defined as death or euthanasia from CHF or worsening clinical signs. Time to treatment failure was significantly longer for the enalapril group (median of 160 days) compared to placebo (87 days).

In a separate trial involving benazepril instead of enalapril, BENCH (BENCH, 1999) enrolled 61 dogs with CHF secondary to MMVD. Dogs received benazepril at a median dose of 0.33 mg/kg PO q24h or placebo, with a 34-week follow-up period. Benazepril was associated with longer time to the primary endpoint of death or withdrawal due to CHF (436 days in benazepril group vs. 151 days in placebo group), and also with higher scores for quality-of-life variables. Together, these studies establish benefits of ACEI in terms of survival, hemodynamics, and quality of life when added to standard therapy for CHF secondary to MMVD.

A critique of these early clinical trials of ACEI in CHF is the absence of pimobendan as part of background therapy, since the drug was not yet available at the time. Some investigators argue that the hemodynamic and survival benefits of pimobendan are sufficiently robust to render RAAS inhibition unnecessary. The VALVE study (Wess et al., 2020) sought to address this question by comparing “dual therapy” (furosemide and pimobendan) with “triple therapy” (furosemide, pimobendan, and the ACEI ramipril) in dogs with MMVD and CHF. The trial did not demonstrate a difference in outcomes between groups. However, several features of the study design limit interpretation (Atkins et al., 2021). Ramipril was administered at a low dose (0.21 mg/kg PO q24h) – the lowest ACEI dose evaluated in a veterinary clinical trial – whereas more recent pharmacokinetic and pharmacodynamic data suggest that an optimal dose is considerably higher (approximately 0.5 mg/kg PO q12h). The average furosemide dose was also unusually high (8 mg/kg/day), making it unsurprising that RAAS suppression with low-dose ramipril alone was inadequate and positive outcomes were not achieved. The high furosemide dose likely also increased the likelihood of aldosterone breakthrough, but spironolactone use was left to clinician discretion and prescribed in fewer than 10% of cases. Finally, over 25% of dogs in the “dual therapy” group had previously received ACEI for an average of 9 months before enrollment, potentially conferring cardioprotective effects prior to randomization.

Taken together, these limitations suggest that the neutral results of VALVE are reflective of study design and dose selection rather than of true absence of benefit from ACEI in CHF. In contrast, the weight of prior evidence supports favorable effects of ACEI on survival, hemodynamics, and quality of life when appropriately dosed. Potential adverse effects of ACEI include hypotension, azotemia, and electrolyte derangements, but these are rare even at the higher doses now recommended (Ward et al., 2021). For this reason, ACEI at a dose of 0.5 mg/kg PO q12h remain a

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recommended component of treatment for stage C MMVD in the ACVIM Consensus Guidelines (Keene et al., 2019).

Clinical Efficacy in Advanced Preclinical Myxomatous Mitral Valve Disease (Stage B2). Two prospective, placebo-controlled clinical trials have evaluated enalapril in dogs with advanced preclinical MMVD, with discordant results. The VETPROOF study (Atkins et al., 2007) enrolled 124 dogs of multiple breeds with MMVD and cardiomegaly, treated with enalapril at a mean dose of 0.46 mg/kg PO q24h. The primary endpoint, delay in time to CHF, showed a modest prolongation of approximately 4 months compared to placebo, with a borderline *P*-value (895 vs. 778 days, $P = 0.06$). Secondary analyses, however, demonstrated significant benefits of enalapril, including a higher proportion of CHF-free dogs at 500 and 1500 days, and improved all-cause survival in a sub-study of 96 participants followed to death (unpublished data). Notably, the survival advantage in this sub-study (approximately 9 months) did not extend specifically to CHF-related mortality, perhaps reflecting ancillary benefits of ACE inhibition such as renal protection.

By contrast, the SVEP trial (Kvart et al., 2002) enrolled 229 Cavalier King Charles Spaniels, including both dogs with and without radiographic cardiomegaly, treated with once-daily enalapril at a lower mean dose (0.37 mg/kg PO q24h). In this population, enalapril had no effect on time to CHF (1150 vs. 1130 days for enalapril vs. placebo), regardless of baseline cardiac size. Given the longer median time to CHF in SVEP, this population overall appears to have had less advanced disease than in VETPROOF, and the single-breed design introduces unique considerations.

Several factors may explain the disparate outcomes of these two studies. VETPROOF enrolled a heterogeneous breed population, whereas SVEP was restricted to Cavalier King Charles Spaniels, a breed with a high prevalence of an ACE gene polymorphism associated with reduced ACE activity (Meurs et al., 2018). This genetic background may blunt the therapeutic effect of ACE inhibition, particularly in the absence of MRAs to block aldosterone escape. Differences in disease severity are also notable: SVEP included both B1 and B2 dogs, while VETPROOF enrolled only dogs with cardiomegaly, more closely reflecting the population at higher risk of progression to CHF. Finally, the lower enalapril dose in SVEP, along with the relatively conservative dosing in both studies, may have limited treatment efficacy. As discussed below, the early ACEI trials in veterinary medicine used doses that are now recognized as suboptimal based on pharmacokinetic, pharmacodynamic, and modeling studies.

A third large-scale study, the DELAY trial, also investigated ACEI in preclinical MMVD, but in combination with spironolactone rather than as a monotherapy (Borgarelli et al., 2020). DELAY was a randomized, placebo-controlled trial of benazepril (median dose 0.3 mg/kg PO q24h) plus spironolactone (median dose 2.8 mg/kg PO q24h) in 184 dogs with stage B2 MMVD. DELAY did not demonstrate benefit in its primary endpoint (onset of CHF or cardiac death), but several secondary endpoints favored active treatment, including reductions in echocardiographic measures of heart size and decreases in NT-proBNP. Because these measures are strongly associated with outcomes in MMVD, the discordance between neutral survival results and positive structural and biomarker changes is difficult to reconcile. Interpretation is also complicated by the low benazepril dose employed (median 0.3 mg/kg PO q24h, lower even than SVEP), raising concern that the ACEI component was subtherapeutic.

A shared limitation of all three trials is the absence of pimobendan, which was not available at the time and has since been shown to provide robust benefit in stage B2 MMVD. Debate therefore continues regarding the role of ACEI in asymptomatic disease. The 2019 ACVIM Consensus Panel could not reach agreement, with 5 of 10 panelists recommending ACEI in stage B2 (Keene et al., 2019). Real-world prescribing data from the LOOK-Mitral study, which aggregates records from 13 North American cardiology specialty centers, similarly suggest that approximately 60% of clinicians routinely prescribe ACEI for stage B2 MMVD (Franchini et al., 2022).

Pharmacokinetics and Pharmacodynamics. In dogs, benazepril, enalapril, and ramipril are ester prodrugs rapidly hydrolyzed in the liver to their active diacid metabolites (benazeprilat, enalaprilat, ramiprilat) (Lefebvre et al., 2007). After oral dosing in healthy dogs, absorption is generally linear

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with dose-proportional pharmacokinetics. The active metabolites are highly protein-bound (>90%) and distribute efficiently to vascular and tissue ACE sites, resulting in prolonged ACE inhibition despite their apparently short elimination half-lives. Very importantly, for the active diacids of ACEI, the apparent terminal half-life in plasma reflects slow dissociation from tissue and endothelial ACE rather than systemic elimination. These small molecules show high affinity for vascular and tissue ACE, creating a large extravascular target compartment. When distribution and clearance are faster than the dissociation rate constant (that is, the rate at which the inhibitor unbinds from ACE), the terminal decline in plasma concentration is governed by unbinding and back-redistribution rather than classical clearance. The result is a prolonged terminal tail and sustained inhibition despite low circulating levels of active metabolites (Toutain, & Lefebvre, 2004).

Benazeprilat exhibits mixed biliary and renal elimination in dogs (~54% biliary, 46% urinary) (SPC Fortekor), often cited as advantageous when renal function is impaired (Toutain et al., 2000); enalaprilat clearance is primarily renal (in humans, >90% recovered in urine within 24 h), making it more susceptible to accumulation with renal impairment (Lefebvre et al., 1999); ramiprilat is eliminated via both bile and urine in dogs (Lefebvre et al., 2006). In contrast, captopril is an active, sulfhydryl-containing ACE inhibitor with rapid systemic clearance in dogs (~600 mL/kg/h) and a short effective half-life (~2–3h) (Singhvi et al., 1981), typically necessitating twice- to thrice-daily dosing; it also has a comparatively higher propensity for gastrointestinal adverse effects (Ismail, 2012). Food effects are minimal for benazepril in dogs (SPC Fortekor). In contrast food reduces captopril bioavailability by ~30–40%, so captopril is best given on an empty stomach (Gordon & Kittleson, 2008).

Quantification of circulating RAAS activity in dogs historically relied on plasma ACE activity (ACEA) as a surrogate. However, measures of ACEA are heavily method-dependent (e.g., use of exogenous substrate, non-physiologic pH/temperature) and reflect catalytic activity rather than *in vivo* peptide concentrations. Importantly, as shown in multiple reports, plasma ACE activity correlates poorly with Ang II and aldosterone, and may indicate "adequate" inhibition of the RAAS even when angiotensins profiling reveals residual RAAS activity during ACE-inhibitor therapy (Jorde, 2002; van de Wal, 2006; Mochel et al., 2013a). The field of RAAS bioanalysis therefore shifted to immunoassays—most commonly plasma renin activity (PRA) and Ang II measured by RIA or ELISA—which brought analyses closer to biology but remained limited by technical issues such as cross-reactivity, matrix effects, and peptide lability (Chappell, 2016). The current standard employs equilibrium liquid chromatography–tandem mass spectrometry (LC–MS/MS) to quantify a peptide panel—Ang I, Ang II, Ang(1–7), Ang(1–5), and aldosterone—following sample stabilization and in-tube equilibrium, yielding a systems-level fingerprint that captures ACE-independent Ang II generation and feedback within the cascade (Pavo et al., 2016). In turn, these richer datasets support mechanism-informed models that better reflect the nonlinearity of the RAAS and the overall pharmacodynamic effects of ACE inhibitors and other RAAS modulators, making findings more clinically relevant (Schneider et al., 2023).

The dose–exposure–response relationship of benazeprilat was recently investigated in healthy dogs using intensive pharmacokinetic sampling, RAAS fingerprinting, and model-based in silico simulations (Sotillo et al., 2023; Schneider et al., 2023). In a 35-day, randomized, partial-crossover study, nine purpose-bred beagles underwent RAAS activation induced by a low-sodium diet and received three benazepril dosing regimens: 0.125 mg/kg every 12 hours (q12h), 0.25 mg/kg q12h, and 0.5 mg/kg every 24 hours (q24h) (Sotillo, 2023). Serial plasma samples collected over 24 hours on intensive sampling days enabled quantification of benazeprilat concentrations alongside a comprehensive RAAS peptide panel, measured via LC–MS/MS. The panel included Ang I, Ang II, Ang III/IV, Ang 1–7, and Ang 1–5, with derived indices such as ACE suppression (ACE-S = Ang II/Ang I), plasma renin activity surrogate (PRA-S), aldosterone suppression (ALT-S), and the aldosterone-to-Ang II ratio (AA2). Benazeprilat exposure increased in a dose-dependent manner across the tested range. Overall, more frequent dosing (q12h) produced greater suppression of Ang II and ACE-S, with reciprocal increases in Ang I and PRA-S. At the highest dose, Ang 1–7 trended

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upward while Ang 1–5 declined, a pattern consistent with ACE inhibition and compensatory activation of the alternative RAAS arm. Hemodynamic variables, including arterial blood pressure, did not differ significantly between groups over the acute 12-hour observation windows. To our knowledge, no comprehensive peptide-based dose–response analyses analogous to Sotillo et al. (2023) have been published for enalapril, ramipril, or captopril in dogs. Existing reports primarily rely on ACE activity, PRA, with limited measurements of angiotensins.

Building on these experimental findings, a nonlinear mixed-effects systems pharmacology model was developed to link benazeprilat exposure with dynamic RAAS peptide changes over time, while accounting for natural oscillations and physiological feedback mechanisms (Schneider et al., 2023). The model jointly fit benazeprilat PK with RAAS biomarker data to reproduce the temporal profiles of Ang I, Ang II, Ang III/IV, Ang(1–7), and Ang(1–5) across doses and dosing frequencies. *In silico* simulations were performed to compare dosing schedules at equivalent daily doses, confirming that twice-daily administration (e.g., 0.25 mg/kg q12h) achieved more sustained Ang II suppression compared to once-daily dosing (e.g., 0.5 mg/kg q24h). Among the tested dosing scenarios, 0.5 mg/kg q12h provided the most robust inhibition of the classical RAAS pathway (Ang II and ACE-S), alongside favorable shifts in alternative-pathway markers (increased Ang 1–7 and decreased Ang 1–5). This systems pharmacology framework has now been deployed as a user-facing virtual trial engine, enabling prospective exploration of dosing regimens, schedule optimization, population variability, and selection of PD readouts (https://benjaminpkpd.shinyapps.io/benazepril-dosage-calculator/). By integrating experimental data with advanced mathematical modeling, this platform provides a powerful tool for optimizing benazeprilat therapy in canine cardiovascular disease, supporting precision dosing strategies to improve therapeutic outcomes in veterinary practice. Further data collection from canine patients with MMVD is needed to refine this mathematical modeling platform and validate these preliminary findings in the context of clinical cardiac disease.

Mineralocorticoid Receptor Antagonists: Spironolactone and Aldosterone Breakthrough

Aldosterone Breakthrough. Aldosterone promotes myocardial and vascular fibrosis, impairs endothelial function, exacerbates electrolyte retention, and potentiates sympathetic and inflammatory signaling. MRA blunt these pathways at the receptor level (genomic and non-genomic), offering benefits orthogonal to ACEI.

Since production of angiotensin II is the major mechanism stimulating release of aldosterone, it might seem that an ACEI alone should be sufficient to decrease aldosterone release and mitigate the long-term harmful effects of aldosterone. However, it is now recognized that aldosterone levels can still be pathologically elevated in some patients receiving ACEI or ARB therapy (alone or in combination). This phenomenon, termed “aldosterone breakthrough,” has been well-characterized in humans (Bomback et al., 2007; Sato & Saruta, 2003) and reported in ACEI-treated dogs with experimental RAAS activation (Lantis et al., 2015, Ames et al., 2015). In one clinical study of 39 dogs with MMVD, approximately 1/3 of dogs demonstrated aldosterone breakthrough after ACEI treatment. Aldosterone breakthrough occurred with approximately equal incidence in dogs with advanced preclinical disease (stage B2) versus historical CHF (stage C) (Ames et al., 2017). Although poorly understood mechanistically, the phenomenon of aldosterone breakthrough demonstrates that single-agent RAAS blockade with an ACEI does not always effectively suppress the negative effects of aldosterone.

Clinical Efficacy in Congestive Heart failure (Stage C). As an MRA, spironolactone mitigates the effects of aldosterone, thereby promoting natriuresis and reducing cardiac fibrosis. In humans, the RALES trial demonstrated a 31% reduction in risk of cardiac mortality for humans with late severe heart failure when spironolactone was added to a loop diuretic, ACEI, and digoxin (Pitt et al., 1999). Additional studies in humans including EPHESUS (Pitt et al., 2003) and EMPHASIS (Zannad et al., 2011) have shown similar benefits with addition of the MRA eplerenone to background treatment in CHF secondary to myocardial infarction. In veterinary medicine, several studies have evaluated use

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of spironolactone in addition to conventional therapy in management of CHF secondary to MMVD in dogs.

The first study of spironolactone in clinical MMVD (Bernay et al., 2010) was a prospective randomized trial following dogs with MMVD that initially participated in one of two short-term studies: (1) a 2-month study with furosemide mandatory at inclusion, and (2) a 3-month study with furosemide not allowed at inclusion. These study groups were then combined for a 12-month follow-up period, in which 123 dogs were initially enrolled and 79 completed the study. Dogs received either spironolactone 2 mg/kg PO q24hr or placebo in addition to background therapy of ACEI, +/- furosemide, and +/- digoxin. The primary endpoint, (a composite of cardiac death, euthanasia, or severe worsening of MR requiring furosemide >10 mg/kg/day or other unauthorized treatment) was reached 11/102 dogs receiving spironolactone and 28/110 dogs receiving placebo (risk reduction of 55%, HR 0.45 [0.22-0.90]). For the endpoint of cardiac mortality, risk reduction was even higher at 69% (HR 0.31 [0.13-0.76]). Overall 15-month survival was significantly higher in the spironolactone group compared to the placebo group (84% vs. 66%). Limitations of this study included the relatively high rate of withdrawal from the study, as well as relatively low event rate, with less than 50% of dogs reaching study endpoints. The relatively long survival time – longer than typically reported for dogs with CHF secondary to MMVD – underscores that fewer than half of the dogs were in CHF at the time of study inclusion. While this study supports the potential benefit of spironolactone in MMVD, the study design – which involved combining groups with different disease stages and variable concomitant treatments – complicates interpretation of this study's findings and limits its generalizability.

The BESST trial (Coffman et al., 2021) randomized dogs with CHF secondary to MMVD to receive either benazepril alone (median dose 0.36 mg/kg PO q24) or a combination of benazepril and spironolactone (benazepril median dose 0.37 mg/kg PO q24, spironolactone median dose 2.97 mg/kg PO q24), in addition to a background of furosemide. The primary outcome variable (cardiac death or euthanasia, worsening pulmonary edema, or worsening of CHF requiring furosemide dose > 8 mg/kg/day) was reached in 168/216 dogs in the benazepril + spironolactone group, and 171/198 dogs in the benazepril group (risk reduction 44%; OR: 0.56 [0.32-0.98]). Median time to reach this cardiac endpoint was 105 days in the combined group vs. 69 days in the benazepril group. Differences in group outcomes were apparent relatively early in the treatment period, with twice as many dogs in the benazepril group reaching the cardiac endpoint by Day 7 compared to the benazepril + spironolactone group (12.6% vs. 6.7%, respectively). Incidence of adverse effects was similar between groups. These results are similar to the outcomes seen in MRA trials in people, demonstrating significant reduction in cardiac morbidity and mortality with the addition of spironolactone to an ACEI in dogs with CHF. The major limitation of this study in terms of clinical generalizability is the absence of pimobendan in the background standard therapy.

One study of spironolactone in canine CHF reported neutral outcomes (Schuller et al., 2010). Eleven dogs with MMVD were included in this study and were randomized to receive low-dose spironolactone (median dose of 0.52 mg/kg PO q24) or placebo, in addition to a background of furosemide, ACEI, +/- pimobendan, and +/- digoxin. No differences in survival, clinical, or echocardiographic variables between groups were noted at 3 or 6 months. Limitations of this study include very small sample size, short follow-up time, and some clinical differences between the groups at baseline. Perhaps most importantly, the dose of spironolactone used was quite low, and below the dose considered optimal by pharmacokinetic and pharmacodynamic studies (see below).

A major goal of the Schuller et al. study was to assess potential adverse outcomes of adding spironolactone in conjunction with other CHF drugs. Similar to the other veterinary trials that showed positive outcomes with spironolactone at higher doses, low-dose spironolactone was also well-tolerated in this context. Two other clinical studies in dog with MMVD have more specifically reported renal function test results and electrolytes before and after addition of spironolactone to background therapy (Lefebvre et al., 2013, Thomason et al., 2007). Both studies found that

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spironolactone was well-tolerated with no clinically relevant changes in renal values or electrolytes compared to either baseline levels (Thomason et al., 2007) or a placebo group (Lefebvre et al., 2013).

Overall, the small number of clinical trials investigating spironolactone in CHF secondary to MMVD meaningful suggest benefit in cardiac morbidity and mortality when added to standard therapy, particularly when combined with an ACEI. Spironolactone is generally well-tolerated, even in conjunction with other CHF therapies. This is consistent with literature in human CHF and supports the synergistic RAAS-suppressing benefit of addressing aldosterone breakthrough. For these reasons, spironolactone is part of current ACVIM consensus guideline recommendations for treatment of CHF secondary to MMVD (Keene et al., 2019).

Clinical Efficacy in Advanced Preclinical Myxomatous Mitral Valve Disease (Stage B2). Only one study, the DELAY trial (Borgarelli et al., 2020), has investigated spironolactone in the preclinical phase of MMVD, and it did so in the context of combined therapy with ACEI. As previously discussed, DELAY showed no benefit of benazepril + spironolactone in its primary endpoint (onset of CHF or cardiac death), but the treatment group did show reductions in echocardiographic measures of heart size and decreases in NT-proBNP. As in earlier ACEI studies, the benazepril dose used was relatively low and may have limited therapeutic impact. If so, the DELAY trial may have functioned more as an evaluation of spironolactone in the absence of robust ACE inhibition, raising the possibility that spironolactone alone is insufficient to meaningfully alter disease course. This interpretation would align with the concept that the principal benefit of MRAs lies in preventing aldosterone breakthrough in patients already receiving effective RAAS blockade, rather than as standalone therapy. In the absence of strong evidence to suggest benefit, spironolactone is not currently recommended by ACVIM consensus guidelines for treatment of stage B2 MMVD (Keene et al., 2019), and prescriber data suggests that only approximately 10% of cardiologists routinely prescribe spironolactone to B2 dogs (Franchini et al., 2022).

Pharmacokinetics and Pharmacodynamics. Regulatory pharmacokinetic studies report rapid biotransformation of spironolactone to canrenone and 7-α-thiomethyl-spironolactone (7α-TMS) in dogs; food increases oral bioavailability (to 80–90%); absorption is approximately linear across 2–4 mg/kg; and steady state conditions are achieved by Day 2–3 (SPC Cardalis®). Reported PK parameters for the active metabolites include large apparent volumes of distribution (153–177 L) (SPC Prilactone®), extensive protein binding (90%) (SPC Cepeloron®), and plasma clearance of 0.9–1.5 L/h/kg, with predominantly fecal elimination (70%) (SPC Prilactone®). With the fixed benazepril/spironolactone combination at labeled doses, terminal half-lives of 6–7 h are reported for the active metabolites (SPC Cardalis®).

In a randomized AB/BA crossover in healthy beagles (7-day periods at 2 or 4 mg/kg q24h; 14-day washout), spironolactone increased circulating aldosterone and classical RAAS peptides—Ang I, Ang II, and Ang-(1–5)—by equilibrium LC–MS/MS. However, separation between the 2 and 4 mg/kg doses was minimal despite higher exposure to canrenone and 7α-TMS at 4 mg/kg. This is consistent with earlier renal Na^+/K^+ antagonism data demonstrating a pharmacodynamic plateau near 2 mg/kg in an experimental hyperaldosteronism model (Masters et al., 2024). These findings also align with data from a furosemide continuous-rate infusion model, in which plasma Ang II and aldosterone increased at 5 hours despite background ACE inhibition (± spironolactone) therapy, indicating robust upstream RAAS activation during forced diuresis (Adin et al., 2021).

Combination Therapy: ACEI and MRA (CARDALIS®)

Clinical Efficacy. Given the synergistic benefits of ACEI and MRA, a combined product has been developed (CARDALIS®) to provide both drugs in a single pill. The fixed combined dose selected during product development was designed to target a dosage of benazepril 0.25 mg/kg PO q24hr and spironolactone 2.0 mg/kg PO q24hr. As discussed, this represents an optimized dose of spironolactone, but potentially a suboptimal dose of benazepril based on retrospective analyses, experimental studies of RAAS endpoints in healthy dogs, and mathematical modeling. The two drugs can also be given separately, allowing for flexibility in dosing between ACEI and MRA.

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As discussed previously in the context of spironolactone, two studies have evaluated the efficacy of the combination of ACEI and MRA in dogs with MMVD. One of these trials (BESST) focused on dogs with newly diagnosed CHF (stage C), and the other trial (DELAY) addressed advanced preclinical disease (stage B2). In the BESST trial (Coffman et al., 2021), 569 dogs with CHF secondary to MMVD were randomized to fixed-dose benazepril/spironolactone (CARDALIS®) versus benazepril alone, in addition to background therapy with furosemide. The CARDALIS® group experienced 44% reduced risk of reaching the primary combined cardiac endpoint (OR: 0.56 [0.32–0.98]) and 27% decreased hazard of cardiac death (HR: 0.73 [0.59–0.89]), with similar adverse events between groups (Coffman et al., 2021).

The DELAY study (Borgarelli et al. JVC 2020) represents the only evaluation of combined ACEI and MRA therapy in preclinical MMVD. Both drugs were prescribed separately rather than as the fixed-dose CARDALIS® product. As noted, DELAY did not show a difference in time to CHF or cardiac death but did report favorable changes in cardiac size and NT-proBNP. Importantly, the spironolactone dose was consistent with current practice, but the low ACEI dose complicates interpretation and makes it difficult to assess the true value of combined therapy in this context.

Pharmacokinetics and Pharmacodynamics. In a prospective, parallel-group study of 18 healthy beagles with RAAS activation induced by a low-sodium diet, three 14-day dosing regimens were compared: (i) labeled dose of Cardalis® q24h (LD24) (benazepril 0.25 mg/kg + spironolactone 2 mg/kg), labeled dose of Cardalis® q12h (LD12), and double-labeled dose of Cardalis® q12h (2LD12) (benazepril 0.5 mg/kg + spironolactone 4 mg/kg) (Manson et al., 2025). Compared with the labeled dose (LD24), 2LD12 most strongly suppressed the classical arm of the RAAS and shifted signaling toward the ACE2/Ang-(1–7) pathway: Ang II and ACE-S decreased, Ang-(1–7) increased, and aldosterone increased relative to Ang II (AA2).

The LD12 group showed intermediate changes. These findings demonstrate that, within the fixed dose combination, ACEI drives circulating Ang II/ACE-S suppression, while MRA contributes receptor-level blockade with expected aldosterone feedback, yielding the strongest overall RAAS modulation when the drug combination is given q12h. Across dosing regimens, there were no clinically relevant changes in creatinine, BUN, sodium, or potassium; while isolated self-limiting gastrointestinal signs (vomiting/soft stool) occurred in a minority of dogs. These observations are consistent with prior reports that ACEI/MRA combinations are generally well tolerated in dogs. Building on previous *in silico* work with ACEI alone, a QSP model is being developed for the benazepril–spironolactone combination, linking active-metabolite exposure (benazeprilat, canrenone, 7-α-thiomethyl-spironolactone) to a multi-analyte RAAS fingerprint (Ang I, Ang II, Ang(1–7), Ang(1–5), aldosterone; indices ACE-S, PRA-S, AA2). The model is designed to deconvolve the net contribution of each agent and support *in silico* optimization of dose/schedule synchronization and biomarker targets for prospective studies (manuscript in preparation).

The observations from the combination study essentially mirrors those from the benazepril monotherapy work using the same experimental model (Sotillo et al., 2023)—greater ACEI yields stronger Ang II and ACE-S suppression with parallel activation of the alternative arm of the RAAS. They are consistent with systems-pharmacology predictions that q12h ACEI provides more sustained classical-pathway control than q24h at the same daily dose. Collectively, these findings support q12h dosing of both small molecules to minimize between-dose gaps, particularly in scenarios of increased RAAS activation (such as loop-diuretic use).

Inodilators

Inodilators combine the drug effects of positive inotropy and vasodilation. In veterinary medicine, the most used drug in this class is pimobendan where most of the evidence for companion animals exists. Other drugs in this category have minimal evidence for use in dogs including levosimendan and milrinone. The inodilator effects of pimobendan and levosimendan are owed to their ability to sensitize the cardiac sarcomere to calcium and inhibit phosphodiesterase-III (Endoh, 2002). With pimobendan and levosimendan intracellular calcium is not increased (Endoh, 2002).

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Milrinone also functions as a phosphodiesterase-III inhibitor and exerts inotropic and vasodilatory effects but does so in part via increased intracellular calcium, distinguishing it from pimobendan (Kittleson, 1991). The increased cellular calcium adds additional risk, resulting in its extremely limited use in settings where pimobendan or levosimendan are available.

Clinical Efficacy in Congestive Heart Failure (Stage C). The QUEST study (Häggström et al., 2008) established that the addition of oral pimobendan to standard CHF therapy was superior to benazepril for treating CHF in canine MMVD. Dogs with MMVD and CHF were randomized to receive either pimobendan at a dose of 0.4-0.6 mg/kg/day or benazepril at a dose of 0.25-0.5 mg/kg/day (Häggström et al., 2008). The study concluded that pimobendan significantly prolonged time to composite endpoint when compared to benazepril (267 vs 140 median days, respectively) (Häggström et al., 2008). When all primary endpoints were considered, median survival time was also significantly longer in the pimobendan group compared to the control group (Häggström et al., 2008). In a later manuscript, (Häggström et al., 2013) subsequent investigation of the data obtained from the QUEST trial found no differences in quality of life between treatment groups but identified a longer time period for dogs treated with pimobendan prior to the first dose escalation of diuretics. Heart size was also reduced in dogs after institution of pimobendan when compared to benazepril (Häggström et al., 2013). These results, although compelling, are limited due to the exclusive enrollment of dogs weighing between 5 and 20kg (Häggström et al., 2008; Häggström et al., 2013). Additionally, they do not address the impact on the common, current practice of combined furosemide, pimobendan, and ace-inhibitor therapy for chronic management of canine CHF secondary to MMVD. Several studies have attempted to investigate the role of furosemide and pimobendan with or without an ace-inhibitor and or spironolactone (Wess et al., 2020; Romito et al., 2025). Limitations such as inappropriately low ACEI dosing and/or retrospective nature of these studies are substantial and significantly limit the ability to interpret the clinical impact of triple or quadruple therapy (Wess et al., 2020; Romito et al., 2025).

Levosimendan use is limited to preclinical studies in canine cardiomyocytes and some studies of animal models (Gao et al., 2023). While results are favorable and mimic those of pimobendan, the driving force for preference of levosimendan in human CHF is secondary to adverse arrhythmia events in humans receiving pimobendan that have not been recapitulated in canine patients receiving pimobendan. Levosimendan is well tolerated and improves a variety of clinical outcomes across a wide number of human CHF etiologies (Mebazaa et al., 2005). These findings taken in combination reinforce the value of inodilators in the management of congestive heart failure across species.

Clinical Efficacy in Advanced Preclinical Myxomatous Mitral Valve Disease (Stage B2). Dogs with advanced preclinical MMVD are typically hyperdynamic via echocardiographic assessment of systolic function, making the use of inodilators less intuitive. This may in part explain the delay between the QUEST and EPIC trials. The EPIC trial (Boswood et al., 2016) was published in 2016 which evaluated the use of pimobendan (0.4-0.6 mg/kg/day) vs. placebo in what is now known as stage B2 MMVD. The primary variable of interest was heart failure free survival days, which were significantly extended by approximately 15 months in dogs receiving pimobendan compared to placebo. Like the QUEST study, a second look at additional data and variable was performed on the EPIC data and identified that pimobendan significantly reduced heart size at Day 35 (Boswood et al., 2018). Like the QUEST trial, the study was limited to small dogs with MMVD, which limits the application of this data to large-breed dogs with the disease (Boswood et al., 2016; Boswood et al., 2018). Additionally, the average dog enrolled in the EPIC trial exceeded the required entrance criteria for left atrial size and left ventricular diastolic dimension. This suggests that later intervention with pimobendan than what was proposed by the EPIC trial may result in similar clinical benefits and warrants further investigation, particularly for clients with financial constraints. The role of pimobendan in patients with cardiorenal syndrome has been investigated in preclinical studies and clinical trials. In experimentally induced MR of healthy laboratory dogs, the administration of pimobendan increased renal blood flow (Kanno et al., 2007). In a clinical trial of dogs with stage B2 MMVD, estimates of GFR via iohexol clearance did not show evidence of improved GFR at standard

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or high (0.5-1.0 mg/kg/day) of pimobendan (Kaplan et al., 2022). Additional investigation into the effect of pimobendan in dogs with concomitant MMVD and renal disease are warranted.

Pharmacokinetics and Pharmacodynamics. Recent pharmacokinetic and pharmacodynamic investigations of pimobendan have focused on multiple routes of administrations and multiple formulations, making generalization of dosing recommendations challenging (Yata et al., 2016; Her et al., 2020; Pichayapaiboon et al., 2021; McManamey et al., 2023). Pimobendan is metabolized to its active metabolite O-demethylated metabolite (ODMP) evidenced by only a small delay in peak pimobendan versus ODMP plasma levels across multiple studies.

In a clinical trial of 34 dogs with stage B2, 14 dogs with stage C, and 8 dogs with stage D MMVD, oral pimobendan pharmacokinetics were investigated using the FDA-approved chewable tablet form of pimobendan, Vetmedin® (McManamey et al., 2023). A sparse sampling and population pharmacokinetic design was performed. Concomitant medications and pimobendan dosage were as prescribed by the attending clinician and not controlled for in this clinical trial, emphasizing the value of this dataset for extrapolation to clinical patients. All dogs were receiving pimobendan at a stable dose (average: 0.36 mg/kg) for at least five consecutive days. In this study, the elimination half-life of the population was approximately 1 and 1.3 hours for pimobendan and ODMP respectively (McManamey et al., 2023). This aligns with the manufacturer-stated elimination half-lives of approximately 0.5 and 2 hours, respectively. The study consistently found a high degree of inter-individual pharmacokinetic variability (>40%) across parameters. In this cohort, that variability could not be explained by disease stage, concomitant therapies, or pimobendan dose differences.

In one single dosing study of eight healthy laboratory dogs receiving a standard dose of 0.25 mg/kg of a non-aqueous pimobendan solution, peak pimobendan and ODMP plasma concentration similarly occurred at 1 and 1.3 hours respectively and remained detectable for 8-12 hours with peak inotropic response by echocardiogram around 3-4 hours post dose (Yata et al., 2016). A pharmacodynamic study of 30 dogs with stage B2 MMVD showed that compared to placebo, 7 days of twice-daily standard (0.25mg/kg) or high-dose (0.5mg/kg) oral pimobendan (Vetmedin®) resulted in echocardiographically determined reductions in heart size and increased systolic function at 3-4 hours post-pill (Kaplan et al., 2022). No pharmacodynamic differences were identified between the standard- and high-dose pimobendan groups; however, the study was small and did not assess outcomes beyond seven days of therapy. (Kaplan et al., 2022). Dose escalation of pimobendan is relatively common in clinical practice and is without clear evidence currently (McManamey et al., 2023).

Loop Diuretics

Clinical Efficacy. Loop diuretics, such as furosemide and torasemide are cornerstone therapies for managing CHF in MMVD. They primarily act by alleviating fluid overload through natriuresis and diuresis. In dogs with MMVD, furosemide is widely prescribed to reduce pulmonary edema and clinical signs of congestion, with evidence from ACVIM consensus guidelines supporting its use in stages C and D (Keene et al, 2019). However, repeated administration of loop diuretics activates the RAAS, contributing to aldosterone breakthrough (Lantis et al., 2015a,b; Ames, 2017; Adin, 2021), diuretic resistance, and potentially the progression of cardiac remodeling. Torasemide, which has a longer duration of action, may offer advantages in cases of furosemide resistance, demonstrating superior diuresis in some canine CHF cohorts (Oyama et al., 2011).

Due to the critical need for diuresis in the management of acute and chronic CHF, placebo-controlled clinical trials supporting its use does not exist in the modern literature. The TORIC study (Cosín et al., 2002) in human patients with CHF established the safety, efficacy and superiority of torasemide when compared to furosemide for management of CHF. The TORIC study established reduced mortality, greater reduction in function heart failure class, and improved electrolyte safety when compared to the furosemide group in more than 1,300 total patients. For veterinary medicine, a parallel pair of randomized, prospective, single-blind, 3-month, positive-controlled studies make up the foundation of the TEST study and provide the best level of evidence and insight to the efficacy

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of furosemide and torasemide in dogs with MMVD (Chetboul et al., 2017). The study also provides comparative data between the parallel studies which generated valuable insight into drug efficacy and potential superiority of torasemide. The TEST study used a median once-daily dose of torasemide at 0.24 mg/kg or a median twice daily dose of furosemide at approximately 1.4 mg/kg. The TEST study aimed to see if the study drugs could impact one of two strata: (1) dogs with ongoing CHF as either a first event or recurrence of uncontrolled congestion requiring a dose adjustment of diuresis, and (2) dogs with controlled CHF. The outcomes of interest in the first uncontrolled CHF stratum were improvement in clinical and radiographic signs of CHF while the outcomes of interest in stratum 2 were maintenance or improvement in clinical status. Importantly, dogs in the TEST study were permitted to receive ACEI, pimobendan and digoxin, provided it was already in place and the dose was not adjusted at enrollment or throughout the 3-month study. However, other medications such as spironolactone or ARB were excluded. The study concluded that torasemide was non-inferior to furosemide at the doses studied and without greater risk of adverse events. A composite endpoint of spontaneous cardiac death, euthanasia due to CHF or worsening of NYHA CHF classification was utilized. Compared to furosemide, torasemide at the dose studied conferred a 2-fold reduction in risk of reaching this composite endpoint. While the TEST study aimed to compare torasemide and furosemide, it represents evidence of efficacy for both compounds which are otherwise challenging to identify within the primary literature, particularly with good sample size.

Pharmacokinetics and Pharmacodynamics. In dogs, oral furosemide has moderate-to-high bioavailability (70–80%) with significant between-dog variability (EMEA/MRL/644/99). Systemic availability is nearly complete after intramuscular administration. After oral dosing, peak plasma concentrations of furosemide are reached within 1–2 hours; the elimination half-life is short (1–3.5 hours) (Koh et al., 2021), findings that are consistent with a rapid onset of action, but a relatively brief diuretic effect (3–6 hours) (Hori et al., 2007). Furosemide is highly bound to plasma proteins (>90%), has a relatively small volume of distribution (0.2–0.7 L/kg) (Koh et al., 2021), and is primarily eliminated by the kidneys (about 60%) (Hirai, 1992). Torasemide offers higher oral bioavailability (typically 90%), a longer half-life (6–7 h), and mixed hepatic/renal clearance, supporting once-daily dosing (commonly 0.1–0.5 mg/kg, with some cases dosed q12–24h) with sustained natriuresis for approximately 12 hours (Uechi et al., 2003; SPC Upcard®; EPAR Isemid®; Pelligand et al., 2020). In CHF, both agents can show reduced diuretic efficacy due to renal impairment and RAAS activation, often necessitating dose escalation; for furosemide, doses may increase up to 4–6 mg/kg q8h, and ACVIM defines refractory (Stage D) cases as requiring >8 mg/kg/day or the torasemide equivalent (Keene et al., 2019).

The dose–response relationship of furosemide was investigated in healthy dogs using intensive sampling and a combined multiple comparison procedures and modeling (MCP-Mod) approach, (Bieth et al., 2016). In a 5-day parallel-group study, 24 healthy beagles were randomized to saline control or furosemide at 1, 2, or 4 mg/kg IM q12h. Blood and urine were collected at steady-state (24 hours post-last dose on Day 4), with PRA measured via enzyme immunoassays and aldosterone via LC–MS/MS. Twenty-four-hour diuresis was quantified from metabolism cage collections. Furosemide induced dose-dependent increases in diuresis, PRA, and aldosterone, with submaximal diuretic effects at doses below those fully activating the circulating RAAS. Projections from the statistical model suggest that doses lower than 1 mg/kg q12 would produce a significant effect on diuresis, with only mild activation of the systemic RAAS in healthy dogs with preserved hemodynamics.

These findings align with broader PK/PD population modeling in healthy dogs, showing dose-dependent diuresis and natriuresis for both furosemide and torasemide, alongside RAAS activation (marked by increased aldosterone levels). Diuretic resistance was reported at high doses; however, a ceiling effect was not reported for either loop diuretic at the maximum tested doses of 8 mg/kg/day for furosemide and 0.4 mg/kg/day for torasemide (Pelligand et al., 2020).

In experimental models of RAAS activation and in clinical studies, furosemide elicits dose-related RAAS activation—with increases in renin activity, Ang II, and aldosterone—despite effective

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diuresis. Accordingly, close monitoring for hypokalemia and pre-renal azotemia is warranted. Transient rises in serum creatinine and urea nitrogen have been reported during aggressive IV regimens, though the magnitude varies by study (Lantis et al., 2015a; Adin et al. 2021).

Adjunctive Therapy

Adjunctive Treatments for Management of Acute Decompensated Congestive Heart Failure

A number of treatments are used by clinicians in the management of acute decompensated CHF secondary to MMVD despite lack of clinical trials demonstrating benefit, in many cases because a placebo-controlled trial would prove unethical. These include oxygen support and advanced applications of oxygen therapy (e.g., high-velocity nasal insufflation of oxygen, mechanical ventilation), paracentesis for treatment of cavitary effusions, and narcotic sedation to reduce anxiety and dyspnea-related distress. Other adjunctive treatments are indicated for patients with specific cardiac complications or comorbidities, such as anti-arrhythmic drugs for patients with hemodynamically significant arrhythmias.

Some pharmacologic therapies for acute CHF have been studied experimentally or in small uncontrolled trials:

- **Nitroglycerin ointment** is a venodilator that can reduce preload and thereby potentially treat pulmonary congestion. An experimental trial in healthy dogs demonstrated splenic dilation in response to nitroglycerine ointment (Parameswaran et al., 1999), but in an experimental model of mitral regurgitation, nitroglycerine ointment did not decrease LV end-diastolic pressure (Nakayama et al., 2007). No clinical trials of nitroglycerine have been performed in dogs with naturally-occurring heart disease.
- **Sodium nitroprusside** is a potent balanced vasodilator that reduces both preload and afterload. It can be titrated as an intravenous infusion with the goal of decreasing severity of mitral regurgitation and improving forward stroke volume. In a canine coronary microembolization model of CHF, nitroprusside decreased pulmonary artery wedge pressure but did not alter cardiac output (Sabbah et al., 1993). Nitroprusside has not been studied in dogs with clinical CHF.
- **Hydralazine** is an arteriolar vasodilator that reduces afterload, again with the goal of decreasing mitral regurgitation and improving forward cardiac output. In a small case series of seven dogs with refractory CHF secondary to MMVD, hydralazine reduced systolic blood pressure and decreased radiographic pulmonary edema (Kittleson et al., 1983). Although no dogs became hypotensive, 6 of 7 dogs developed sinus tachycardia. In another small study, 22 dogs with MMVD and early CHF were assigned to either enalapril or hydralazine monotherapy, followed by the addition of furosemide 3 weeks later (Haggstrom et al., 1996). Dogs receiving hydralazine, but not enalapril, had increased heart rate and decreased heart size compared to baseline; although blood pressure was not measured in this study, hypotension was cited as a potential mechanism to explain these findings.
- **Clevidipine** is a novel intravenous dihydropyridine calcium channel blocker that can acutely reduce afterload with the goal of reducing mitral regurgitation and improve cardiac output. Like sodium nitroprusside, clevidipine can be administered as a constant-rate infusion to titrate systemic blood pressure and reduce afterload on a minute-to-minute basis. In a prospective, randomized, open-label clinical trial—partially published in abstract form and with the full report in preparation—clevidipine was well tolerated in dogs with MMVD and CHF. Clevidipine at a median dose of 5.25 μg/kg/min achieved a target reduction of mean arterial pressure by 20% in a predictable and dose-dependent fashion without major adverse events or impact on measures of renal function.
- **Dobutamine** is a β1 agonist and positive inotrope that improves cardiac contractility and cardiac output, and in the setting of MMVD might function as a pharmacologic annuloplasty to decrease mitral regurgitation. Like nitroprusside, it is short-acting and can be titrated as an infusion;

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disadvantages include the risk of tachyarrhythmias and relatively short period of effectiveness due to downregulation of β1 receptors. In a coronary microembolization model of canine CHF, dobutamine increased cardiac output and left ventricular ejection fraction, and decreased systemic vascular resistance (Sabbah et al., 1993). Dobutamine has not been evaluated in naturally-occurring canine heart disease.

Adjunctive Treatments for Management of Chronic Refractory Congestive Heart Failure (Stage D)

Many pharmacologic therapies are used for adjunctive treatment in late disease stages despite lack of evidence-based recommendations. Clinical trials are challenging in populations with such late-stage disease due to low patient numbers, lack of homogeneity of the population, comorbidities, and ethical concerns in medically fragile patients. The following outlines existing evidence for some of these adjunctive therapies. As noted above, additional treatments may be indicated in patients with specific cardiac complications, particularly anti-arrhythmic drugs for ventricular rate control in atrial fibrillation (e.g., diltiazem, digoxin) or for control of frequent or complex ventricular ectopy (e.g., sotalol, mexiletine, amiodarone):

- **Amlodipine** is a dihydropyridine calcium-channel blocker and arterial vasodilator. It is clearly indicated in dogs with MMVD and concurrent systemic hypertension, but it may also have adjunctive benefits in normotensive dogs by reducing afterload and thereby decreasing the severity of mitral regurgitation. In an experimental model of MR, amlodipine significantly decreased left atrial pressure (Suzuki et al., 2012), and in a small, short-term, non-blinded echocardiographic study of naturally occurring MMVD, treatment was associated with reduced left atrial and left ventricular dimensions (Park et al., 2022). No blinded or placebo-controlled trials have been performed. A retrospective study of 21 dogs with CHF due to MMVD described amlodipine administered alongside furosemide, pimobendan, ACEI, and spironolactone, suggesting that long-term combination therapy is well tolerated (de Madron et al., 2011).
- **Sildenafil** is a phosphodiesterase V inhibitor that serves as a selective pulmonary vasodilator. Pulmonary arterial hypertension is common in advanced MMVD, arising either from passive postcapillary overload from elevated left atrial pressure, or from a combination of postcapillary and reactive precapillary mechanisms. Sildenafil is often recommended in cases of MMVD complicated by clinically significant pulmonary hypertension, particularly when echocardiographic evidence is strong (Reinero et al., 2020) and compatible clinical signs are present (e.g., syncope and dyspnea despite adequate control of left-sided CHF, or presence right-sided CHF). In a small double-blinded placebo-controlled crossover study in 13 dogs with pulmonary hypertension secondary to MMVD, sildenafil improved exercise capacity and quality of life scores compared to placebo, with no adverse effects (Brown et al., 2010). Echocardiographically-estimated pulmonary artery pressure decreased in both groups, and while the change was numerically larger in the sildenafil group, the difference was not statistically significant. Several retrospective studies have also reported positive outcomes of sildenafil in larger cohorts of dogs with pulmonary hypertension that include subsets with MMVD (Kellum & Stepien, 2006; Bach et al., 2006). A commonly cited concern is that pulmonary vasodilation might worsen pulmonary edema; however, a randomized placebo-controlled trial in 14 dogs with MMVD, CHF, and moderate postcapillary pulmonary hypertension found sildenafil to be well tolerated without exacerbation of pulmonary edema (Saetang et al., 2020). Two small trials have evaluated sildenafil in preclinical MMVD (>75% stage B1) dogs: one suggested improved heart rate variability (Pirintr et al., 2017), while the other reported modest reductions in some echocardiographic variables and NT-proBNP (Kijtawornrat et al., 2017). However, these differences were small, often remained within normal ranges, and were inconsistent across timepoints, underscoring the difficulty of assessing pharmacologic interventions in early preclinical MMVD.
- **Hydrochlorothiazide** is a thiazide diuretic that blocks the sodium–chloride cotransporter in the distal convoluted tubule and may be added for sequential nephron blockade when loop diuretics

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are insufficient. Evidence is limited to experimental studies in healthy dogs (Kusumoto et al., 1973); it has not been evaluated in naturally occurring MMVD or CHF.

- **Cough suppressants (e.g., hydrocodone)** are often prescribed for symptomatic relief of cough, especially in dogs with concurrent dynamic airway disease. In patients with advanced MMVD, cough may be exacerbated by compression of the left mainstem bronchus secondary to left atrial enlargement. There are no specific studies of cough suppressants in dogs with MMVD or CHF.
- **Bronchodilators (e.g., theophylline, aminophylline, and terbutaline)** are sometimes prescribed to relieve bronchoconstriction and reduce cough. As weak sympathomimetics, they may also modestly increase cardiac contractility and heart rate. No studies have specifically assessed bronchodilators in dogs with MMVD or CHF.

Emerging Therapies

Angiotensin Receptor–Neprilysin Inhibitors (ARNI). Neprilysin is a broad-spectrum endopeptidase that degrades vasoactive peptides, including natriuretic peptides (ANP/BNP), bradykinin, and substance P. Sacubitril, a neprilysin inhibitor, increases natriuretic peptide concentrations, while valsartan blocks the AT1 receptor. The fixed-dose combination (sacubitril/valsartan) is thus designed to shift the neurohormonal status toward vasodilation, natriuresis, and antifibrosis without the adverse safety profile typically associated with ACE–NEP dual inhibition (Gu et al., 2010; Menendez, 2016). In dogs, preclinical evidence from the low-sodium model of RAAS activation demonstrates that sacubitril/valsartan increases circulating cGMP and reduces aldosterone levels compared to ACEI or ARB monotherapy (Mochel et al., 2014; Mochel et al., 2018). These preclinical findings were subsequently supported by pilot clinical data. In a double-blind, placebo-controlled pilot trial involving thirteen dogs with Stage B2 MMVD, 30 days of sacubitril/valsartan therapy (20 mg/kg PO q12h) attenuated the increase in the urinary aldosterone-to-creatinine ratio compared with placebo, without significant effects on NT-proBNP concentrations, echocardiographic parameters, or thoracic radiographic findings (Newhard et al., 2018). In Stage C MMVD, a 4-week randomized study comparing sacubitril/valsartan (20 mg/kg PO q24h) with ramipril (0.125 mg/kg PO q12h) (both added to standard care including furosemide and pimobendan) demonstrated greater potential for reverse remodeling—reflected by reductions in LA/Ao, LVIDDN, EDVI, and ESVI—while NT-proBNP and urinary aldosterone levels were comparable between groups (Saengklub et al., 2021). Collectively, these findings indicate that ARNI effectively modulate RAAS and natriuretic pathways in dogs, potentially mitigating aldosterone breakthrough and cardiac remodeling. However, the lack of large-scale, long-term outcome trials in naturally occurring canine MMVD underscores the need for further research to establish their role relative to ACEI/MRA standards of care.

Sodium Glucose Cotransporter-2 Inhibitors (SGLT-2I). Multiple large, placebo-controlled trials have established SGLT-2I as disease-modifying therapies across heart failure phenotypes in humans, with benefits extending well beyond glycemic control (Zinman et al., 2015; Neal et al., 2017; Persson et al., 2018; Birkeland et al., 2019; McMurray et al., 2019; Inzucchi et al., 2020; Packer et al., 2020; Butler et al., 2021; Kosiborod et al., 2023). Mechanistically, these small molecules reduce oxidative stress and chronic inflammation, promote autophagy, attenuate advanced glycation end-product signaling, and shift myocardial and whole-body substrate use toward ketone bodies (Bertoccini & Baroni, 2021). SGLT-2 inhibition also modulates innate immunity: in endothelial cells and macrophages, dapagliflozin suppresses TLR-4 expression and downstream NF-κB activation and promotes polarization toward anti-inflammatory M2 macrophages (Abdollahi et al., 2022). This property complements RAAS inhibition, which primarily attenuates neurohormonal and hemodynamic stress, by adding direct immunometabolic benefits that further mitigate cardiac and renal injury.

Although peer-reviewed outcome data in dogs remain limited, early translational studies support their tolerability and potential use as adjunctive therapy in CHF. In a rapid atrial pacing model of canine atrial fibrillation, canagliflozin limited atrial electrical/structural remodeling and fibrosis (Nishinarita et al., 2021). The pharmacokinetics and nonclinical safety of empagliflozin in

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dogs are well characterized. In beagles, empagliflozin shows low clearance, high oral bioavailability and high selectivity (Grempler et al., 2012; Chen et al., 2015). In 52-week dog studies, toxicological findings included dose-related vacuolation of the adrenal zona glomerulosa and renal changes observed at exposures far exceeding clinical levels; the adrenal effect was considered secondary to glucosuria-related osmotic/diuretic stress (Bogdanffy et al., 2014). Overall, these nonclinical data suggest a favorable safety margin relative to projected therapeutic exposures, with renal toxicity anticipated at supratherapeutic empagliflozin doses.

Pilot clinical studies further demonstrate feasibility and short-term safety in client-owned dogs. In naturally obese dogs, DWP16001 (another SGLT-2I drug candidate) (0.2 mg/kg PO q24h) reduced body weight, body condition score, and anthropometric measures over 8 weeks relative to controls (Rhee et al., 2022). As add-on therapy to insulin in diabetic dogs, DWP16001 improved glycemic control in a pilot study (An et al., 2024), and a subsequent multicenter randomized controlled trial of DWP16001 (0.025 mg/kg PO q24h) as an adjunct to insulin in diabetic dogs demonstrated that reductions in glycemic markers were significantly greater in dogs with poor baseline control (fructosamine ≥ 500 µmol/L, HbA1c ≥ 6%), with a trend toward decreased insulin requirement without clinically relevant adverse effects (An et al., 2025). In insulin-treated diabetic dogs, add-on canagliflozin (2–4 mg/kg q24h for 7 days) significantly lowered interstitial glucose versus insulin alone but increased the frequency of hypoglycemia, warranting a reduction in the insulin dose (Box et al., 2024).

A recently published randomized pilot in five dogs with symptomatic stage C MMVD found that short-term dapagliflozin (0.25–0.45 mg/kg once daily) improved cardiac geometry and function versus conventional therapy, with reductions in left-atrial and left-ventricular dimensions over the treatment window—consistent with a favorable hemodynamic effect (Saengklub et al., 2025). Additionally, two preliminary studies, not yet published in peer-review form, have investigated SGLT-2I medications in dogs with clinical heart disease. The first study was a small double-blind, placebo-controlled 30-day pilot in dogs with DCM. Empagliflozin (0.3 mg/kg q24h) was well tolerated, increased β-hydroxybutyrate levels and induced glycosuria, but did not produce short-term changes in echocardiographic indices (Pla et al. 2024). The second was an open-label pilot study involving 10 dogs with stage B2 or C MMVD or DCM. Dapagliflozin (0.5-0.8 mg/kg PO q24h for 5-7 days), added to stable background therapy, induced significant glycosuria and decreased urine potassium and creatinine (publication under review).

Collectively, these preliminary findings position SGLT-2I as promising adjunct to existing therapies for MMVD, with the potential to lessen diuretic burden and improve metabolic efficiency. However, their clinical utility remains to be validated in large, well-controlled cohorts of dogs with CHF to establish efficacy, safety, and optimal dosing strategies.

Glucagon-Like Peptide-1 Receptor (GLP-1R) Agonists. GLP-1R agonists exert anti-inflammatory and antioxidant effects that extend beyond the treatment of obesity and T2DM. GLP-1 receptor agonist peptide analogs modulate endothelial and immune-cell signaling, attenuate NF-κB–associated pathways and reduce pro-inflammatory mediators; in human patients they have been shown to improve vascular function and lower circulating endothelial activation markers (e.g., sICAM-1, sVCAM-1) (Krasner et al., 2014; Wei et al., 2016; Alharbi 2024). In human cardiac tissue, higher GLP-1R expression correlates with low-grade inflammation and endothelial dysfunction. Increased GLP-1R expression was associated with higher oxidative stress that was attenuated by GLP-1R agonists via the canonical GLP-1R–AMPK pathway (Cordeanu et al., 2023). Among patients with T2DM and CKD, semaglutide lowers the risk of major kidney events as well as cardiovascular and all-cause mortality (Perkovic et al., 2024). GLP-1R is expressed in renal and immune compartments, and GLP-1R agonists attenuate pro-inflammatory and pro-fibrotic signaling in both clinical populations (Tuttle et al., 2023) and experimental models of diabetic kidney disease or hypertension (Alicic et al., 2021; Dalbøge et al., 2022; Alicic et al., 2023).

In dogs, cardiac GLP-1 research has focused on pacing-induced DCM and ischemia–reperfusion models aimed at human translation, while much of the remaining canine literature centers on

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metabolic rather than cardiac endpoints. In pacing-induced DCM and coronary occlusion/reperfusion models, short-term GLP-1 (GLP-1(7-36) and its metabolite GLP-1(9-36)) infusions increased myocardial glucose uptake, improved left-ventricular performance, and limited post-ischemic stunning, with mechanistic links to p38α MAPK–NO signaling and enhanced L-type Ca^{2+} current at the myocyte level (Nikolaidis et al., 2004, 2005; Bhashyam et al., 2010; Xiao et al., 2011). In metabolic studies, liraglutide stabilized post-prandial glycemia in healthy and type 1 diabetic dogs without increasing insulin, consistent with prandial glucagon suppression; chronic exenatide reduced body weight and improved β-cell function indices in prediabetic dogs but did not enhance whole-body insulin sensitivity or glucose tolerance (Ionut et al., 2016). Intraportal exenatide failed to show insulin-independent hepatic effects in conscious dogs (Edgerton et al., 2013). Finally, prolonged, physiologic GLP-1 exposure can indirectly increase hepatic glucose uptake in canine models (Dardevet et al., 2004), and preliminary data suggest that liraglutide facilitates weight loss and appetite control in obese senior dogs (Dik et al., 2025).

Conclusion

Evidence-based therapy for MMVD in dogs has advanced considerably, particularly with respect to RAAS-modulating drugs and loop diuretics, where multiple clinical trials demonstrate improved survival, quality of life, or both. Nonetheless, controversies persist regarding the role of RAAS inhibition in preclinical disease, the optimal dosing strategies for RAAS modulators, and the integration of newer therapies into standard practice. While emerging data from pharmacokinetic/pharmacodynamic studies and translational models are beginning to refine dosing regimens and support precision approaches, large-scale clinical outcome studies remain limited. For the practicing clinician, current recommendations in advanced preclinical MMVD (ACVIM stage B2) emphasize the use of pimobendan and critical weighing of potential pros and cons of RAAS inhibition. In symptomatic disease, the evidence-based standard of care is a synergistic combination of loop diuretics, pimobendan, and multimodal RAAS blockade. Looking forward, the incorporation of novel drug classes and individualized medicine may provide further gains in survival and quality of life. Continued clinical research will be essential to fill existing gaps and to ensure that therapy for canine MMVD is both evidence-based and optimized for individual patients.

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