OSTEOARTHRITIS

A randomized clinical efficacy study targeting mPGES1 or EP4 in dogs with spontaneous osteoarthritis

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Canine studies of spontaneous osteoarthritis (OA) pain add valuable data supporting drug treatment mechanisms that may translate to humans. A multicenter, randomized, double-blind, placebo- and active-controlled study was conducted in client-owned dogs with moderate OA pain to evaluate efficacy of LYA, an inhibitor of microsomal prostaglandin E synthase-1 (mPGES1), an EP4 antagonist (LYB), and carprofen, versus placebo. Of 255 dogs screened, 163 were randomized (placebo/LYA/LYB/carprofen: n = 43/39/42/39) and 158 completed treatment. Efficacy versus placebo was assessed using Bayesian mixed-effect model for repeated measure analyses of the Canine Brief Pain Inventory (CBPI) pain interference score (PIS; primary endpoint), pain severity score, and overall impression, as well as the Liverpool Osteoarthritis in Dogs (LOAD) mobility score. The posterior probability that the difference to placebo was <0 at week 2 was 80% for LYA and 54% for LYB for CBPI PIS (both <95% predefined threshold). For secondary endpoints, the posterior probability that the difference to placebo was <0 at week 2 ranged from 89 to 96% for LYA and from 56 to 89% for LYB. The posterior probabilities comparing carprofen to placebo groups were ≥90% for all efficacy endpoints. The proportion of dogs with one or more adverse event was not significantly different from placebo (32.6%) for LYA (35.9%) or carprofen (25.6%), but the rate for LYB (59.5%) was higher versus placebo (P = 0.017). LYA treatment demonstrated consistent improvement in all efficacy measures, suggesting that inhibition of mPGES1 may be an effective treatment for chronic pain associated with OA.

INTRODUCTION

Osteoarthritis (OA) is the most common joint disorder in humans, characterized by progressive inflammation and degeneration at the articular surface (*1*, *2*). Clinically, this results in stiffness, swelling, pain, and progressive disability (*3*). Spontaneously occurring chronic OA in dogs occurs as a consequence of various conditions, including hip and elbow dysplasia, after bone and ligament injuries, as well as with articular degeneration and inflammation associated with aging (*4*, *5*). OA occurs in about 20% of adult dogs and 14% of adult humans (*6*, *7*), and the prevalence increases with age (*6*). The high prevalence and debilitating nature of OA in dogs and in humans make this disease a considerable cause for concern for both veterinary and human medicine.

Chronic pain is a hallmark of OA in both humans and dogs (*6*, *7*). Existing preclinical models of pain have poor predictive validity for efficacy in humans (*8*, *9*). An ideal animal model should share similar characteristics of human disease, such as genetic basis, anatomy and physiology, pathological response(s) and underlying pharmacological mechanism(s), and measurable phenotypic endpoints (*9*). The ideal animal model would also be responsive to drugs with known clinical efficacy and be predictive of clinical efficacy (improvement or resolution) (*9*). Companion dogs with spontaneous OA pain meet most of these criteria (*10*–*13*), acknowledging that the genetic basis of OA is not completely understood in canines or humans. Canine OA pain can be measured using validated scales such as the Canine Brief Pain Inventory (CBPI) (*14*). The CBPI is an owner-completed scale that was developed to parallel the Brief Pain Inventory used in human studies and is validated to collect the same domains, severity of pain, and the impact of pain on function in companion dogs with OA (*14*–*16*).

The similarity and prevalence of canine and human OA, coupled with the availability of validated pain scoring tools, make naturally occurring OA in dogs an attractive parallel disease state in which to study new molecular entities. Canine clinical studies designed for translational purposes may have greater predictive validity for human efficacy than induced preclinical models. Data from interventional canine studies provide support for human clinical studies and could accelerate the development of new therapies while also addressing unmet animal health needs (*17*).

Inflammation contributes to the progressive pain and debility of OA (*18*). Humans and canines with OA respond similarly to anti-inflammatory drugs, including nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) and selective cyclooxygenase-2 (COX-2) NSAIDs that target prostaglandin E (PGE) (Fig. 1) (*19*, *20*). Nonselective NSAIDs successfully reduce pain and inflammation via the depletion of prostaglandin H and the subsequent elimination of PGE. A consequence of this approach is that common homeostatic prostaglandins other than PGE are also depleted, giving rise to gastrointestinal (GI) side effects, which often limit NSAID use (*21*, *22*). Although COX-2–selective NSAIDs have reduced GI side effects in humans (*23*), they have been less effective in mitigating GI side effects in canines (*20*). In addition, there is concern that these drugs may increase major adverse cardiovascular events in humans as a consequence of thromboxane and prostacyclin imbalance (*24*, *25*). Selective mechanisms that reduce formation of PGE while avoiding depletion of prostaglandin H and other downstream "housekeeping" prostaglandins may help mitigate some of these safety concerns.

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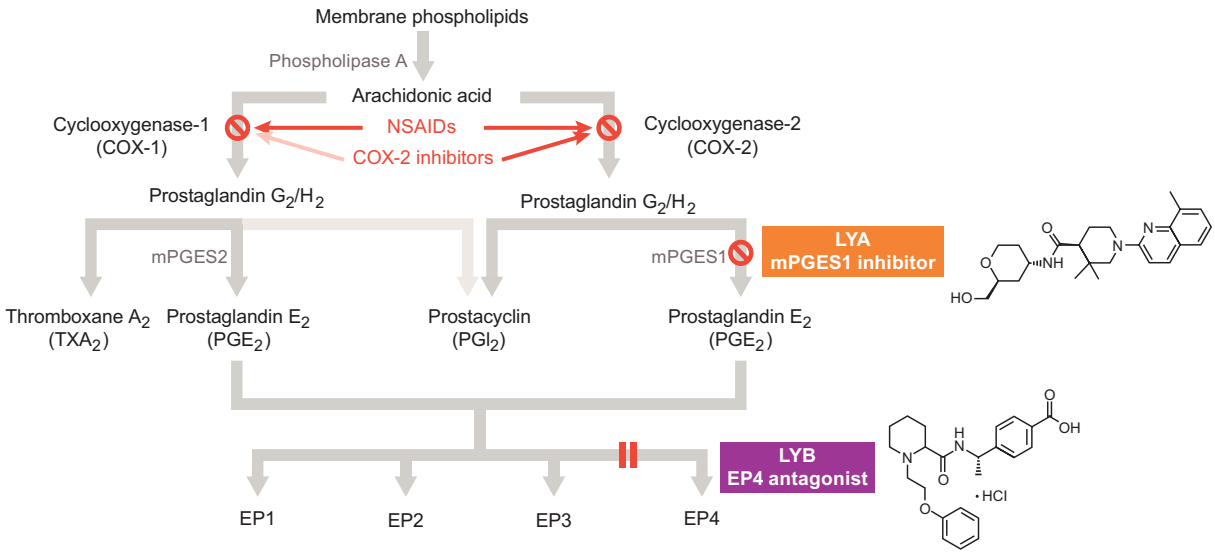

Fig. 1. Targeting the prostaglandin E pathway in osteoarthritis. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and inflammation via the depletion of prostaglandin H and the subsequent elimination of prostaglandin E_2 (PGE_2). LYA directly reduces the production of PGE_2 by inhibiting microsomal prostaglandin E synthase-1 (mPGES1). PGE_2 exerts its biological effects by binding to four specific membrane-bound G protein–coupled receptors (EP1 to EP4). LYB is a specific antagonist of the EP4 receptor (indicated by || in the figure). EP, E-type prostanoid; LYA, mPGES1 inhibitor; LYB, EP4 antagonist.

PGE_2 inhibition is thought to be the primary mechanism by which these drugs exert their anti-inflammatory and analgesic effects (*1*, *26*). Microsomal PGE synthase-1 (mPGES1), the terminal synthase responsible for the synthesis of PGE_2, is a well-recognized target for the development of new anti-inflammatory agents (*27*, *28*). The first selective mPGES1 inhibitors have entered clinical trials (*29*, *30*).

PGE_2 exerts its biological effects by binding to four specific membrane-bound G protein–coupled receptors [EP1 (E-type prostanoid 1) to EP4]. Of the four receptors, EP4 is associated with the development of inflammation and autoimmunity and is not known to be associated with the direct modulation of other prostanoids (*31*). In 2016, a PGE_2 EP4 receptor antagonist (grapiprant) was approved for use in dogs, representing the first demonstration of efficacy for this new class of piprant drugs (*32*–*34*). However, there continues to be a need for alternative treatments to alleviate chronic pain that have a more favorable safety profile than the majority of existing drugs.

We present the results of a proof-of-concept study for the efficacy of two molecules belonging to the mPGES1 inhibitor (LYA) and EP4 antagonist (LYB) drug classes (Fig. 1). These molecules have not previously been tested in clinical trials in humans or animals with spontaneous pain. The purpose of this study was to assess how the efficacy of three compounds, including carprofen as a positive control, which act at different points in the PGE_2 pathway, would differ. We hypothesized that either the elimination of PGE_2 production or antagonism of one of its receptors would be the mechanism by which NSAIDs exert their analgesic effects. This canine study was designed similarly to human OA studies in the effort to leverage the clinical utility of these molecules for chronic OA pain in human and/or animal health, using Bayesian methodology.

RESULTS

Study population

Of the 255 adult companion dogs screened with moderate OA pain, 163 were randomized to treatment and 158 (96.9%) completed the treatment period (Fig. 2). The first dog was enrolled on 2 October 2015, and the last dog completed the in-life phase of the study on 11 October 2016. Two (1.2%) dogs discontinued because of adverse events (AEs), and one dog in the LYB treatment group died (was euthanized because of unrecoverable illness). One dog in the LYB group discontinued treatment due to owner-perceived therapeutic ineffectiveness. Compliance with study drug administration in the treatment groups was as follows: placebo, 86.0%; LYB, 88.1%; LYA, 92.3%; carprofen, 97.4%.

The treatment groups were well balanced in terms of baseline demographics (Table 1). The mean age was 9.3 years, and weight was evenly balanced [lighter (15 to 32 kg) and heavier (>32 to 50 kg) weight categories] between the four treatment groups. Most dogs had been spayed or neutered. There were significantly fewer spayed females in the carprofen group compared with the placebo group ($P = 0.0396$). Given the relatively small number of dogs in this clinical trial and the numeric and percentage distribution of dogs of each sex across all groups, this difference was not considered clinically relevant. The most frequent breed represented in all treatment groups was "large mixed breed." Labrador Retriever, Golden Retriever, and German Shepherd were the most frequent pure breeds. A selection of baseline OA severity measures was also similar among treatment groups, as assessed by the CBPI pain interference score (PIS), pain severity score (PSS), overall assessment, and the Liverpool Osteoarthritis in Dogs (LOAD) mobility (Table 1).

Efficacy

Mean (SD) CBPI PIS decreased (improved) after 2 weeks of treatment, and improvements, albeit of lesser magnitude, were still evident for each treatment group at the end of the follow-up period (Fig. 3A). Only the least squares mean change from baseline LOAD mobility score in the carprofen group was significant versus placebo at week 2 ($P = 0.011$; Fig. 4). The 95% credible regions for the change from baseline in CBPI PIS were less than 0 for all treatment arms both at the end of treatment and at the end of the follow-up

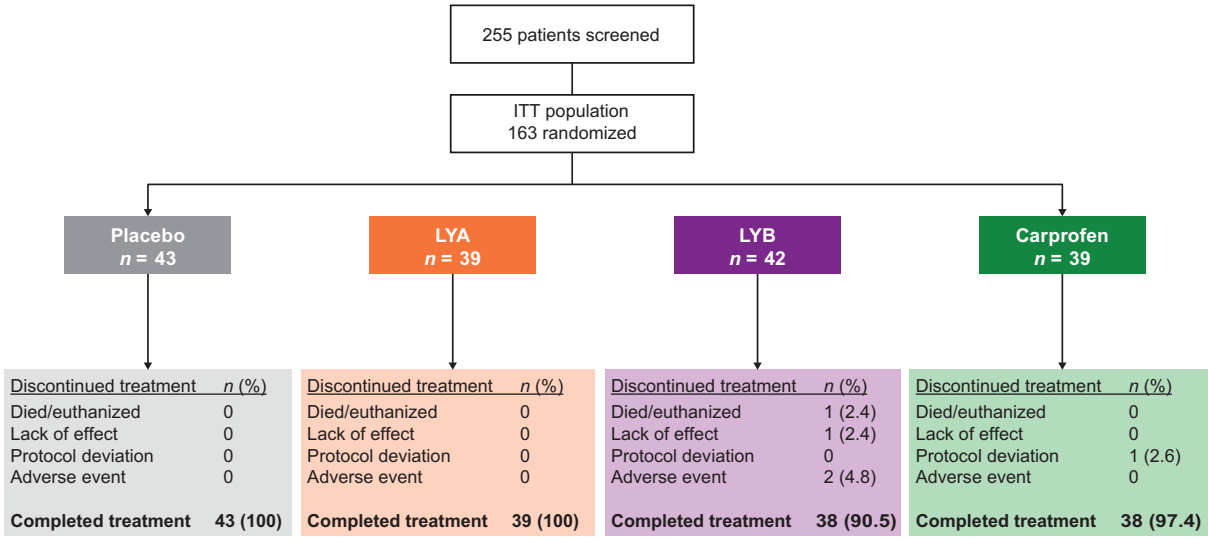

Fig. 2. Patient disposition. Of the 255 adult companion dogs screened with moderate osteoarthritis pain, 163 were randomized to treatment and 158 (96.9%) completed the treatment period. ITT, intent-to-treat.

period. At the end of treatment, the posterior probability that the difference to placebo was less than 0 for the primary endpoint of CBPI PIS was 80 and 54% for LYA and LYB, respectively; therefore, neither treatment achieved the predefined 95% threshold for declaring superiority. The posterior probability for the difference between carprofen and placebo at the end of treatment was 93%, which also fell short of the superiority threshold (Table 2).

Improvements from baseline in each of the secondary endpoints of CBPI PSS, CBPI overall impression, and LOAD mobility were observed in all treatment groups at week 2, and the respective 95% credible regions did not include 0. The posterior probabilities for the differences of LYA to placebo and carprofen to placebo for the secondary CBPI endpoints at the end of treatment ranged from 89 to 94%, whereas the posterior probabilities for the differences of LYB to placebo at week 2 were 78% (CBPI PSS) and 56% (CBPI overall impression). For LOAD mobility, the posterior probabilities for the differences of LYA to placebo and carprofen to placebo at week 2 exceed the 95% threshold, 96 and 99%, respectively, and the posterior probability for the difference of LYB to placebo at week 2 was 89% (Table 2).

Dogs were followed for 2 weeks after discontinuation of study drug to evaluate safety and efficacy signals after withdrawal. After 2 weeks off study drug, all improvements had lessened, yet most of the 95% credible regions for CBPI PSS and LOAD mobility remained below 0.

The proportion of CBPI PIS responders after 2 weeks of treatment was similar to the LYA and carprofen treatment groups (44% each; n = 17 dogs in each group; table S1). CBPI PIS responder status was observed in 26% of dogs receiving LYB (n = 11) and 35% of dogs receiving placebo (n = 15). Differences were not statistically significant versus placebo for any treatment group and were not considered clinically relevant.

Rescue therapy was administered to three dogs in the placebo group, none in LYA, one in LYB, and one in the carprofen group, with no significant difference among treatment groups. All instances of rescue therapy were administered after the dogs had completed study treatment (between visit 3 and visit 4) and therefore did not affect data collection for the primary or secondary endpoints.

Pharmacokinetics

Table 3 summarizes the key pharmacokinetic (PK) parameters of LYA and LYB. For both compounds, maximum plasma drug concentrations were achieved at about 3 hours after dose and then declined with a half-life of about 9 hours. The steady state was anticipated to be achieved after 2 days of dosing. The steady-state drug exposures were compared with historical potency estimates from dog whole blood target engagement assays (specific for each LY; see the "Dose selection" section), which suggested that through the 12-hour dosing interval, all dogs receiving LYA had plasma concentrations above IC_{90} (drug concentration to achieve 90% of the maximum inhibition effect) and most dogs receiving LYB (94%) had plasma concentrations above IC_{80}. Hence, the administered doses of both LYA and LYB resulted in desired drug exposure, per study protocol.

Safety

The proportion of dogs with one or more treatment-emergent AE (TEAE) was not significantly different between the placebo group and the LYA group or the carprofen group, but the proportion was significantly greater in the LYB group versus placebo (P = 0.017; Table 4). Two dogs in the LYB group experienced one or more serious AE (SAE). One dog experienced SAEs of allergic edema, conjunctivitis, and lethargy, and one dog experienced acute multiorgan failure, was euthanized, and was found to have a large neoplasm (pheochromocytoma). The investigators categorized the relationships to study treatment as "possible" for the SAE multiorgan dysfunction (with pheochromocytoma) and "probable" for the SAE allergic edema. Three dogs in the LYB group discontinued because of an AE, accounted for by the two dogs with SAEs and an additional dog with a TEAE of diarrhea. The diarrhea was considered mild in severity, lasted 1 day, and resolved without medical intervention. The most frequently occurring TEAEs were in the system organ class "digestive tract disorders" (placebo, 16%; LYA, 26%; LYB, 26%; carprofen, 15%), and the most frequently reported TEAEs across the treatment groups were diarrhea and emesis (Table 5).

A table of abnormal laboratory parameters occurring in ≥5% of dogs in any treatment group is available in the Supplementary

Table 1. Baseline demographics and disease characteristics. Data are means (SD) unless stated otherwise. Pairwise comparisons between treatments and placebo were not significant ($P > 0.05$), unless otherwise indicated. F, female; LOAD, Liverpool Osteoarthritis in Dogs; M, male.

	Placebo (*n* = 43)	LYA (*n* = 39)	LYB (*n* = 42)	Carprofen (*n* = 39)	Total (*N* = 163)
Age, year	9.8 (2.8)	9.2 (2.8)	9.0 (3.1)	8.9 (3.3)	9.3 (3.0)
Weight, *n* (%)					
15–32 kg	24 (55.8)	21 (53.8)	23 (54.8)	21 (53.8)	89 (54.6)
>32–50 kg	19 (44.2)	18 (46.2)	19 (45.2)	18 (46.2)	74 (45.4)
Gender, *n* (%)					
F, spayed	25 (58.1)	21 (53.8)	26 (61.9)	16 (41.0)*	88 (54.0)
F, intact	0	0	0	1 (2.6)	1 (0.6)
M, neutered	15 (34.9)	15 (38.5)	16 (38.1)	22 (56.4)	68 (41.7)
M, intact	3 (7.0)	3 (7.7)	0	0	6 (3.7)
Breed, *n* (%)					
Large mixed breed	12 (27.9)	11 (28.2)	13 (31.0)	17 (43.6)	53 (32.5)
Labrador Retriever	8 (18.6)	6 (15.4)	9 (21.4)	7 (17.9)	30 (18.4)
Golden Retriever	4 (9.3)	3 (7.7)	4 (9.5)	3 (7.7)	14 (8.6)
German Shepherd	4 (4.9)	4 (10.3)	1 (2.4)	1 (2.6)	10 (6.1)
CBPI pain interference	5.2 (2.0)	4.8 (2.0)	5.1 (2.3)	5.2 (2.1)	5.1 (2.1)
CBPI pain severity	4.2 (1.8)	4.1 (1.9)	4.2 (1.9)	4.4 (1.9)	4.2 (1.9)
CBPI overall impression, *n* (%)					
Excellent	1 (2.3)	2 (5.1)	4 (9.5)	3 (7.7)	10 (6.1)
Very good	11 (25.6)	10 (25.6)	16 (38.1)	7 (17.9)	44 (27.0)
Good	22 (51.2)	19 (48.7)	14 (33.3)	23 (59.0)	78 (47.9)
Fair	9 (20.9)	7 (17.9)	7 (16.7)	6 (15.4)	29 (17.8)
Poor	0	1 (2.6)	1 (2.4)	0	2 (1.2)
LOAD mobility	24.0 (5.7)	23.7 (4.9)	23.1 (6.0)	25.6 (5.6)	24.1 (5.6)

*$P = 0.040$ versus placebo.

Materials (table S2). No significant differences were observed in hepatic values (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, or γ-glutamyltransferase) or renal values (blood urea nitrogen and creatinine) among the treatment groups. Increased platelet count was significantly greater in the LYB- and carprofen-treated groups compared with placebo. Increased cholesterol was significantly greater in the carprofen-treated group compared with placebo- and LYB-treated groups.

DISCUSSION

PGE_2 and its downstream effectors are potential targets for modulation of inflammation associated with OA (*31*, *34*). This randomized, double-blind, placebo- and active-controlled, multicenter clinical study investigated the efficacy of an mPGES1 inhibitor (LYA) and an EP4 antagonist (LYB) for the treatment of chronic OA pain and was designed to inform drug development for both human and animal health.

This proof-of-concept study was designed with a robust criterion for success based on the primary endpoint measure and supplemented with additional secondary pain measures. Although none of the tested compounds met the criterion for success, the totality of all pain endpoints measured suggests a proof-of-concept efficacy signal for mPGES1 inhibition as an alternative to NSAIDs. LYA was also well tolerated. In this study of dogs with spontaneous OA, a threshold of 95% probability of superiority to placebo with the CBPI PIS was prespecified. Although the mPGES1 inhibitor (LYA) had an 80% probability of superiority for CBPI PIS, it is important to note that LYA approached or exceeded the 95% threshold for the secondary endpoints, CBPI PSS (94%) and LOAD mobility score (96%). In addition, 44% of LYA-treated dogs met the CBPI responder criteria after 2 weeks of treatment. Together, these collective observations across scales and responder calculations suggest an efficacy signal for this mPGES1 inhibitor.

In this study, the EP4 antagonist (LYB) did not demonstrate consistent or significant separation from placebo for the primary and secondary endpoints. After this study was initiated, a manuscript summarizing the efficacy of the EP4 antagonist grapiprant was published (*34*), and it has since been approved for treatment of dogs with OA. This study and the grapiprant study used the same CBPI response criteria (PIS reduction from baseline ≥2 and PSS reduction from baseline ≥1) at week 2. In the grapiprant study, 41% of grapiprant-treated dogs versus 28% of placebo-treated dogs fulfilled the CBPI response criteria. In our study, 44% of LYA

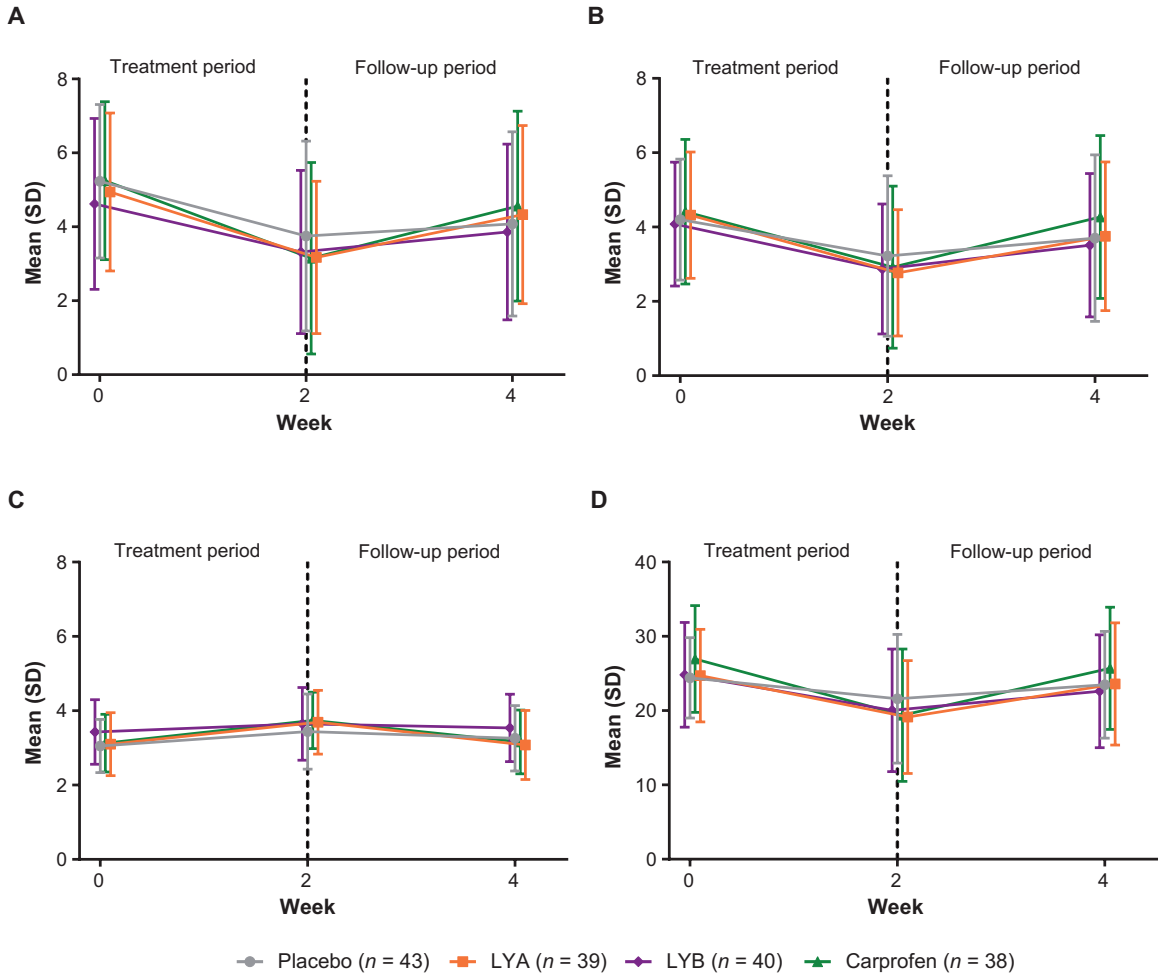

Fig. 3. Primary and secondary endpoints. (**A**) Canine Brief Pain Inventory (CBPI) pain interference, (**B**) CBPI pain severity, (**C**) CBPI overall impression, and (**D**) LOAD mobility scores. All data are means (SD).

(mPGES1)- and carprofen-treated dogs versus 35% of placebo-treated dogs were responders, whereas only 26% of those receiving LYB (EP4) were responders. It is not entirely clear why LYB did not demonstrate efficacy in this study; however, it is useful to reflect on the assay used to estimate EP4 antagonism. Because there is no direct measure of EP4 antagonism, we, like others (*35*, *36*), used the tumor necrosis factor α (TNFα) release assay as a proxy. Our assumption was that efficacy would be achieved with the TNFα assay at the IC_{50}. However, wide variability in the assay has been reported (*36*) and was seen in our tests in dog blood. This presents challenges in correlating TNFα release with efficacy. One might also expect that as the selectivity of the compound increases, the efficacy window would shrink compared with nonselective NSAIDs that can interact with several components of the pathway. Moving forward, a direct measure of occupancy, and perhaps a compound with a wider safety margin, would be required to detect a reliable efficacy signal. Head-to-head studies would also be required to fully understand why the EP4 antagonist used in this study did not show similar results to those observed with grapiprant.

Pain is a multidimensional condition, and the type of measure used may detect different aspects of pain. In this study, the LOAD mobility scale showed better separation from placebo than did the CBPI. Because the LOAD and CBPI have inherent differences in their design, it is possible that the differences in score could reflect the multidimensional aspects of pain. The LOAD was used as the primary screening measure for pain to minimize the bias on the primary measure (CBPI), a technique that has been used in other studies. Therefore, it is also possible that the greater separation observed with the LOAD scale may underscore the importance of using techniques to minimize inflated values due to bias at screening.

We hypothesized that selective mechanisms targeting mPGES1 or EP4 would avoid some of the undesirable side effects associated with nonselective NSAIDs and COX-2–selective NSAIDs. In this study, LYA was generally well tolerated; the incidence of dogs with one or more TEAE was not significantly different between placebo and LYA or carprofen. The incidence of TEAEs was significantly higher for LYB than for placebo. The most frequently occurring AEs across treatment groups were GI related and were reported in 21% of dogs. This was not statistically different from placebo for any of the treatment arms. GI AEs are common with the use of non-selective and COX-2–selective NSAIDs and also for grapiprant (*34*, *37*). However, whereas the GI effects caused by NSAIDs can be serious and result in mucosal ulceration (*33*, *38*, *39*), these events are not anticipated with mPGES1 inhibition or EP4 antagonism.

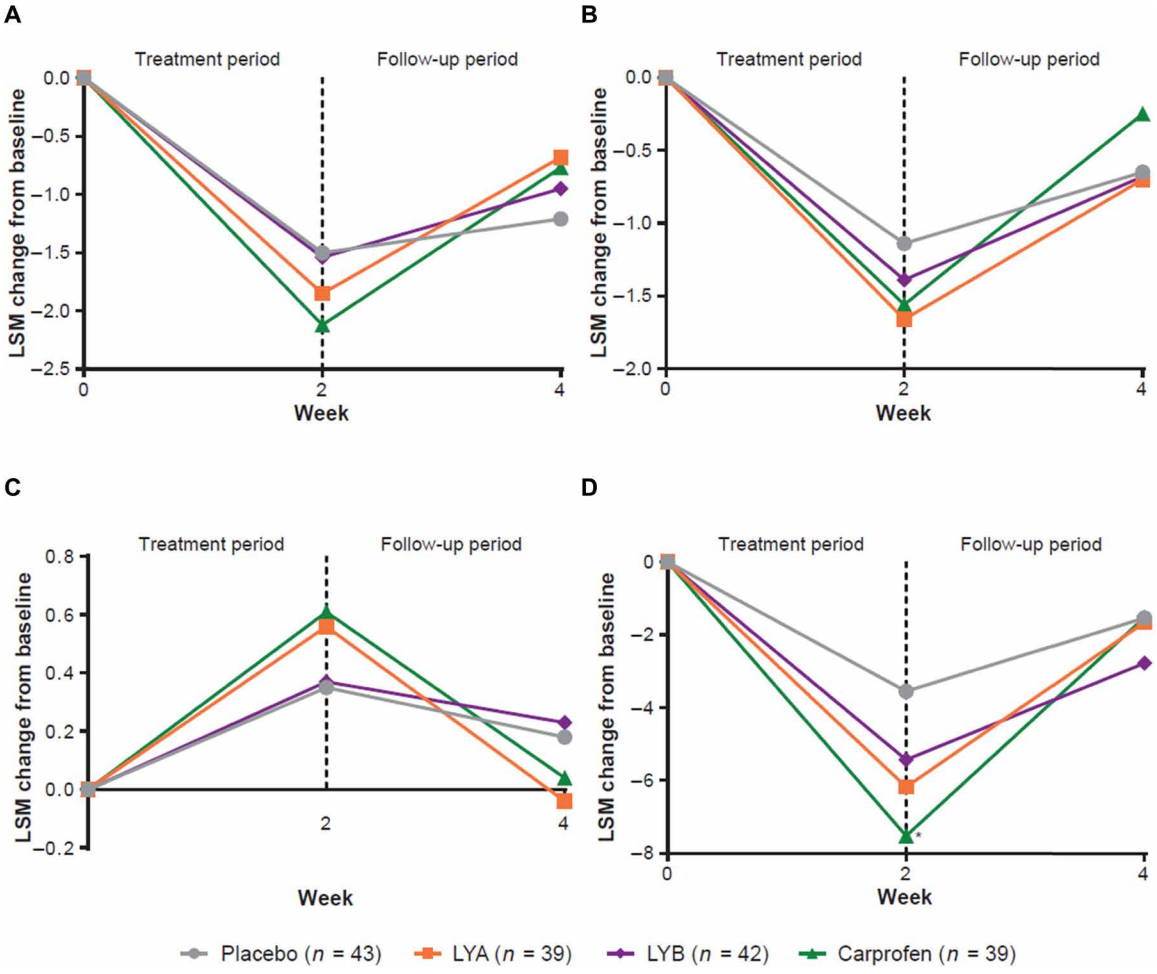

Fig. 4. Change from baseline CBPI domains. (**A**) CBPI pain interference, (**B**) CBPI pain severity, (**C**) CBPI overall impression, and (**D**) LOAD mobility. At week 2, placebo, $n = 43$; LYA, $n = 39$; LYB, $n = 40$; carprofen, $n = 38$. At week 4, placebo, $n = 43$; LYA, $n = 39$; LYB, $n = 39$; carprofen, $n = 38$. Changes from baseline in the active treatment groups were not statistically different from placebo in any of the CBPI domains by MMRM analyses of the full analysis set. *$P = 0.011$ for carprofen versus placebo by MMRM analyses of the full analysis set. *LSM*, least squares mean; *MMRM*, mixed-effect model for repeated measures.

Clinical laboratory results were monitored for safety and did not demonstrate statistically significant changes between groups for hepatic or renal measures. Platelet count was significantly higher in dogs treated with LYB compared with placebo; however, the clinical relevance of this is not clear. The comparatively undesirable safety profile of LYB could be a consequence of dose selection for LYB. We opted to use a high dose for the detection of an efficacy signal, which could have compromised the anticipated safety profile. As with other drugs in development, further definition of the safety profile is necessary once effective analgesia is fully established.

A follow-up period was included in this study to continue to monitor safety after study drug discontinuation and to observe effect on the efficacy scales. During the follow-up period, there were no AEs attributable to study drug, and the CBPI and LOAD values trended toward baseline values, as expected. However, because owners were aware that their dog was not receiving any treatment after 2 weeks, the results of the owner-completed outcome measures at 4 weeks should be interpreted with this in mind.

The retention of enrolled canine subjects on this study is notable. Discontinuation of patients for nonmedical (not safety-related) reasons is a challenge, especially in human clinical trials, and can result in imputation of missing data. In this study, between 90 and 100% of dogs in all treatment groups returned for the endpoint (visit 3). No discontinuations were due to "lost to follow-up," a classification used when the subject does not return and cannot be reached for data collection. Many factors contribute to retention of subjects in both human and animal clinical studies: intrinsic study design aspects, perceived or actual subject burden, and site-related factors. This study was, by intent, relatively short and with only four required visits. However, we hypothesize that another factor in the high retention rate seen in this study is related to the level of altruism among dog owners who chose to enroll their dog in this clinical trial and the attention to detail by the participating veterinary clinical trial sites.

Studies with spontaneously occurring disease in animals are a recognized tool for proof-of-concept evaluation and translation of data to relevant human conditions. These studies can facilitate the identification of promising drug candidates that have greater potential to emerge as successful medications (*40*). The role that companion dogs with OA can play in therapeutic development has been

Table 2. Probability of superiority to placebo in the change from baseline after 2 weeks of treatment and 2 weeks of follow-up for the efficacy endpoints. Bayesian MMRM model: change from baseline = baseline + treatment + visit + baseline * visit + treatment * visit + site + body weight, with unstructured variance-covariance structure for a dog's measurements at end of treatment and after 2 weeks of follow-up. ITT population. *n*, number of evaluable canines at week 2; PIS, Pain Interference Score; PSS, Pain Severity Score.

Endpoint	Placebo (*n* = 43)	LYA (*n* = 39)	LYB (*n* = 42)	Carprofen (*n* = 39)
CBPI PIS				
Week 2, *n*	43	39	40	38
Posterior mean (95% credible region)	−1.5 (−1.99, −1.01)	−1.8 (−2.35, −1.34)	−1.5 (−2.05, −1.04)	−2.1 (−2.64, −1.61)
Probability of superiority to placebo	–	80%	54%	93%
Week 4, *n*	43	39	39	38
Posterior mean (95% credible region)	−1.2 (−1.63, −0.80)	−0.7 (−1.10, −0.25)	−1.0 (−1.38, −0.53)	−0.8 (−1.19, −0.34)
Probability of superiority to placebo	–	6%	23%	10%
CBPI PSS				
Week 2, *n*	43	39	40	38
Posterior mean (95% credible region)	−1.14 (−1.53, −0.75)	−1.66 (−2.06, −1.25)	−1.39 (−1.79, −0.98)	−1.56 (−1.97, −1.16)
Probability of superiority to placebo	–	94%	78%	90%
Week 4, *n*	43	39	39	38
Posterior mean (95% credible region)	−0.65 (−1.00, −0.31)	−0.70 (−1.05, −0.34)	−0.68 (−1.04, −0.32)	−0.25 (−0.61, 0.11)
Probability of superiority to placebo	–	57%	54%	8%
CBPI overall impression				
Week 2, *n*	43	39	40	38
Posterior mean (95% credible region)	0.35 (0.14, 0.56)	0.56 (0.34, 0.78)	0.37 (0.15, 0.59)	0.61 (0.39, 0.83)
Probability of superiority to placebo	–	89%	56%	93%
Week 4, *n*	43	39	39	38
Posterior mean (95% credible region)	0.18 (−0.01, 0.37)	−0.04 (−0.24, 0.15)	0.23 (0.03, 0.43)	−0.04 (−0.16, 0.23)
Probability of superiority to placebo	–	8%	6%	19%
LOAD mobility				
Week 2, *n*	43	39	40	38
Posterior mean (95% credible region)	−3.55 (−5.36, −1.74)	−6.17 (−8.05, −4.31)	−5.43 (−7.29, −3.58)	−7.52 (−9.42, −5.63)
Probability of superiority to placebo	–	96%	89%	99%
Week 4, *n*	43	39	39	38
Posterior mean (95% credible region)	−1.53 (−2.90, −0.16)	−1.65 (−3.06, −0.24)	−2.77 (−4.18, −1.36)	−1.52 (2.93, −0.10)
Probability of superiority to placebo	–	54%	87%	50%

Table 3. Summary of key pharmacokinetic parameters for LYA and LYB. The pharmacokinetics of LYA and LYB were analyzed with conventional noncompartmental analysis methods to obtain C_{max} (maximum drug concentration), C_{trough}, and t_{max} (time of maximum observed drug concentration) at steady state. They were also analyzed with population PK analysis methods based on a one-compartment model with first-order absorption to obtain PK parameters such as CL/F (apparent clearance) and V/F (apparent volume of distribution) and to derive parameters such as $t_{1/2}$ and $AUC_{ss,24hr}$ (area under the concentration versus time profile at steady state over 24 hours). As reference, the IC_{90} (drug concentration to achieve 90% of the maximum inhibition effect) of the in vitro target engagement assay was 113 ng/ml for LYA, and the IC_{80} of the in vitro target engagement assay was 864 ng/ml for LYB. $C_{trough,ss}$, predose concentration at steady state.

Parameter based on noncompartmental analysis	LYA (n = 39)	LYB (n = 42)
	Estimate	Estimate
Steady-state C_{max} (ng/ml)*	722 (67.0%)	7930 (75.5%)
Steady-state $C_{trough,ss}$ (ng/ml)*	436 (77.8%)	3080 (68.2%)
Steady-state t_{max} (hours)†	3.13 (0.85–5.33)	3.07 (0.75–4.68)
Parameter based on population PK analyses	**Population estimate**	**Population estimate**
CL/F (liters/hour)‡	3.12 (2.66–3.71)	7.18 (5.45–8.42)
V/F (liters)‡	39.5 (35.5–50.2)	89.3 (34.7–141)
$t_{1/2}$ (hours)§	8.78	8.62
$AUC_{ss,24hr}$ (mg·hour/liter)‖	15.3 (38.7)	115 (35.0)

*Reported as geometric mean [geometric %CV (percent coefficient of variation)]. †Reported as median (range). ‡Reported as mean (95% confidence interval). §Derived using the population estimate of CL/F and V/F based on the following equation: log(2)/(CL/V). ‖Reported as geometric mean (%CV), calculated using the post hoc estimate of CL/F of each patient, following the equation dose/(CL/F).

previously explored and suggested to have translational potential (*12*). Companion dogs with OA pain have many similarities to humans with OA pain, from clinical presentation and pathophysiology, to the pharmacological mechanisms considered effective. Therefore, comparative medical studies to evaluate new mechanisms are expected to improve the probability of predictive translation for human efficacy. The use of comparative medical studies in early proof of concept is anticipated to reduce the cost and time taken to get drugs to market compared with traditional pharmaceutical development models (*13*, *17*, *40*), and will expand potential therapeutic opportunities to be developed for animal health.

Consistent performance of the carprofen active control group demonstrated that the study design and execution were appropriate to detect analgesic activity. The magnitude of improvement in CBPI PIS and PSS after 2 weeks of carprofen treatment (posterior mean change from baseline, −2.1 and −1.6, respectively) was comparable to improvements in similar estimates previously reported in dogs with OA of the forelimb or hindlimb, treated with carprofen for the same duration (PIS and PSS median change from baseline, −1.1 and −1.2, respectively) (*41*). Estimates for placebo also appear to be within expectations based on other published data for clinical studies in companion dogs (*42*). Response to placebo can be influenced by expectation bias (by the owner, in this study), by regression to the mean, and potentially by increased attentiveness to behavior changes, much like in human studies (*43*). The use of multiple outcome measures to confirm clinical improvement can increase the confidence in study results (*44*). Multiple scales also support study construct validity and provide insights into the multidimensionality of OA pain.

PK analysis suggested that projected target exposure concentrations were achieved for both LYA and LYB. Through each dosing interval at steady state, all dogs receiving LYA had plasma concentrations above IC_{90} and most dogs receiving LYB had plasma concentrations above IC_{80}. Compliance with study drug was high (86 to 97% across all four treatment groups), suggesting that most dogs were adequately dosed and adhered to the treatment protocol. The potential rationale for lack of consistent separation from placebo for LYB, despite adequate exposure concentration in this study, is not clear and would require further investigation.

Limitations of this study include its relatively small sample size. The population size was selected on the basis of the number required for detection of an efficacy signal for either LY compound compared with placebo while not exposing an excessive number of dogs to experimental drugs during an early proof-of-concept study. Less common AEs cannot be detected in the small samples sizes or duration of observation used in this study. Other published canine studies of slightly longer duration include efficacy measurements at or soon after 1 month of treatment (*34*, *37*). For a chronic condition, longer studies are required to inform safety and efficacy beyond the 2-week treatment period in this study. However, the short duration we used was intentional because our purpose was only to detect an efficacy signal to support our assumptions before advancing development in human clinical trials or further development in dogs. Although this study did not include pharmacodynamic measurement of PGE_2, based on the mechanistic understanding of carprofen and of mPGES1 inhibition, reduction of PGE_2 was the presumed mechanism for the mPGES1 inhibitor effect.

Another limitation of this study is that it relied entirely on subjective pain scales that were completed by the owner, based on their observations for the last 7 days. Similar scales are used in human studies, and the CBPI scale was modeled after the human Brief Pain Inventory scale. These scales provide integrated human insight of multiple observations of the subject's behavior across time. Alternative objective measures for the assessment of pain and functional improvement in OA, such as force platform gait analysis, electrodermal activity, actimetry, and quantitative sensory testing (*7*, *45*–*47*), could be used as complementary assessments in future trials to remove observer bias. Continuous actimetry quantifies movement. However, it would likely also need documentation of potential confounders such as the presence or absence of other animals, people, or activities in the household, which may stimulate movement that may not be attributable to the level of pain. Gait analysis is a useful tool to provide a time point evaluation of limb and body movement and weight placement and could be especially useful when the animal is well adapted to the environment where the testing is done. Each of these measures may provide additional and different insights of the complex condition of pain. Technology advancements with objective tools such as these could make these measures increasingly useful for canine clinical trials where the animals are managed in their owner's homes.

In conclusion, although the primary endpoint success criterion was not met for any of the tested compounds, based on the observation

Table 4. Adverse event summary. Pairwise comparisons between treatments and placebo were not significant ($P > 0.05$) unless otherwise indicated. Data are *n* (%). AE, adverse event; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

Incidence, *n* (%)	Placebo (*n* = 43)	LYA (*n* = 39)	LYB (*n* = 42)	Carprofen (*n* = 39)
Patients with ≥1 TEAE*	14 (32.6)	14 (35.9)	25 (59.5)†	10 (25.6)
≥TEAE in system organ class of digestive tract disorders	7 (16.3)	10 (25.6)	11 (26.2)	6 (15.4)
Patients with ≥1 SAE‡	0	0	2 (4.8%)§	0
Discontinued due to ≥1 AE‖	0	0	3 (7.1%)¶	0
Died/euthanized	0	0	1 (2.4)	0

*$P = 0.017$ versus placebo. †Assessed during the treatment period. ‡Assessed over the duration of the study, including follow-up. §SAEs were allergic edema, conjunctivitis, and lethargy in one patient and multiorgan failure, neoplasm, and death in another patient. ‖At any point over the duration of the study, including follow-up. ¶Discontinuations were due to the SAEs listed above and due to diarrhea in an additional patient.

Table 5. Treatment-emergent adverse events with frequency of >5% in any treatment group. Pairwise comparisons between treatments and placebo were not significant ($P > 0.05$). NOS, not otherwise specified.

Preferred term, *n* (%)	Placebo (*n* = 43)	LYA (*n* = 39)	LYB (*n* = 42)	Carprofen (*n* = 39)
Emesis	3 (7.0)	6 (15.4)	6 (14.3)	3 (7.7)
Diarrhea	5 (11.6)	5 (12.8)	5 (11.9)	2 (5.1)
Polydipsia	2 (4.7)	3 (7.7)	6 (14.3)	2 (5.1)
Anorexia	1 (2.3)	3 (7.7)	5 (11.9)	1 (2.6)
Lethargy	1 (2.3)	3 (7.7)	1 (2.4)	1 (2.6)
Polyuria	1 (2.3)	0 (0)	3 (7.1)	1 (2.6)
Bacterial skin infections	1 (2.3)	0 (0)	0 (0)	2 (5.1)
Eye disorder NOS	0 (0)	0 (0)	3 (7.1)	0 (0)
Pruritus	0 (0)	2 (5.1)	0 (0)	0 (0)

of the totality of data across several measures, this study suggests a proof-of-concept pain efficacy signal for the mPGES1 inhibitor, LYA, when given orally at a dose of 0.75 mg/kg twice a day in companion dogs with OA pain. LYA treatment resulted in improvements in clinical signs of pain compared with placebo for several endpoints and of magnitude comparable to carprofen treatment. Further, LYA was generally well tolerated, with incidence of AEs comparable to placebo. This further supports that mPGES1 inhibition is a useful approach for effective analgesia. The EP4 antagonist LYB did not demonstrate consistent efficacy versus placebo, the reasons for which need further investigation. Although LYB did not perform better than placebo on all endpoints in this study, EP4 antagonism is still considered an important mechanism for pain relief and warrants further investigation. This study demonstrates that the inhibition of mPGES1 with LYA represents a promising new target for the treatment of OA pain in both humans and animals.

MATERIALS AND METHODS

Study design

This was a randomized, double-blind, placebo- and active-controlled trial designed to assess the efficacy and safety of LYA and LYB versus placebo in the treatment of dogs with chronic pain due to OA. The study comprised three periods: screening, treatment, and follow-up. The screening period (visit 1 to visit 2; 1 to 2 weeks in length) was followed by a 2-week treatment period (visit 2 to visit 3), at the end of which the primary efficacy endpoint was assessed (visit 3). A 2-week follow-up period (visit 3 to visit 4) was conducted after the last dose of study drug was administered.

Dogs recruited for this study were privately owned male and female (neutered/spayed or intact) companion pets. Care was taken to select dogs that had already received adequate care and appeared to have had humane treatment in the home environment. Owners were required to be at least 18 years of age. For inclusion in the study, dogs had to be at least 2 years of age at screening, be generally healthy with no evidence of serious chronic disease other than OA, and weigh 15.0 to 50.0 kg, and female dogs should not be pregnant. Each dog had a history consistent with OA pain for at least 6 months, physical examination findings consistent with appendicular OA pain, and radiographic confirmation of OA. At screening, dogs were also required to have a LOAD mobility score of ≥13 but ≤46, which is a middle range for the scale, and extrapolated to represent moderate pain. LOAD was included as an entry criterion to minimize score inflation bias on CBPI (the primary endpoint). At randomization, dogs were required to have a LOAD mobility total score of ≥13 and ≤46. In addition, because the CBPI PIS was the primary endpoint, the score must not have decreased by more than three points from visit 1 to visit 2. This criterion was included to eliminate dogs with severe inflation bias to CBPI PIS at screening, or highly variable score reporting across time, before treatment. Dogs that did not meet these criteria were excluded.

Dogs were also excluded if they had other primary sources of pain, known or suspected soft tissue injury or bleeding disorders, or other orthopedic or neurological disease. Any medication or herbal preparation used for pain control, any systemic corticosteroids, or intra-articular injections were excluded from use during the study. In addition, to be enrolled in the study, certain medications must not have been given for a certain time before visit 1. Nonselective NSAIDs, COX-2–selective NSAIDs, and short-acting corticosteroids were not administered for at least 2 weeks and intermediate/long-acting corticosteroids were not administered for at least 4 weeks before screening. Use of intra-articular injections or stem cell therapy was not allowed for 6 months before screening. Dogs were also excluded if they had a known lack of tolerance, allergy, or nonresponse

to any NSAID or had received physical therapy for orthopedic indication during or ≤1 week before baseline. Routine preventive medications and procedures were to be avoided between visit 2 and visit 3. Dog food containing nutraceuticals, or other components thought to be favorable to OA, was allowed, provided that there was consistency in type and amount of food for at least 1 month before baseline and it was to remain the same throughout the study.

All dog owners gave signed informed consent before the start of any study procedure. The study protocol was approved by and conducted in accordance with the Lilly-Elanco Institutional Animal Care and Use Committee (IACUC) and by IACUC committees at each of the institutional investigational sites. The study was conducted according to the principles of Good Clinical Practices as outlined by the Veterinary International Conference on Harmonization (*48*). Preclinical safety for dogs was evaluated prior to this clinical trial.

The primary objective of this study was to evaluate the efficacy of LYA and LYB versus placebo in the treatment of dogs with chronic OA pain, as measured by the change from baseline to week 2 in the CBPI PIS. The secondary objectives of this study were to compare the efficacy of LYA, LYB, and carprofen relative to placebo as measured by the change from baseline to week 2 in the CBPI PSS and overall impression, as well as LOAD mobility. Additional secondary objectives were to evaluate the PK of LYA and LYB and to assess the safety of all active treatments relative to placebo, and LY drugs relative to carprofen, by assessing the incidence of AEs and changes in clinical laboratory measures. In addition, the proportion of CBPI responders, defined as dogs with CBPI PIS reduction from baseline ≥2 and CBPI PSS reduction from baseline ≥1 at visit 3, was also assessed. Frequency of rescue therapy use was also recorded in each treatment group.

The primary objective would be achieved if there was at least a 95% probability that the change from baseline in the CBPI PIS at week 2 was smaller for either of the LY treatment groups relative to placebo. The sample size of 40 dogs per treatment group was based on the primary endpoint, with assumptions for this study based on previous studies that reported CBPI results (*22*, *23*). The sample size provided about 90% probability to achieve the primary objective, if at least one LY drug had a true treatment improvement of 0.8 points relative to placebo on CBPI PIS. This assumed a between-dog SD of 1.2 points. There was only a 9% probability of achieving the primary objective if the effects of both LYA and LYB were similar to placebo.

This study was conducted at seven veterinary academic centers and private practices in the United States, in privately owned dogs. After meeting all enrollment criteria, dogs were assigned (1:1:1:1) by computer-generated randomization code to placebo, LYA, LYB, or an active control (carprofen). Randomization was stratified by body weight (low, 15 to 32 kg; high, >32 to 50 kg) and site to ensure even representation across the four treatment groups. Study investigators and dog owners were blinded to treatment assignment throughout the study. To preserve the blind, only a minimum number of personnel at the central drug dispensing unit had access to the randomization schema and codes before study completion. All study drugs were identical in appearance, and all dogs within a specific weight range grouping were administered the same number of capsules regardless of assigned treatment.

The first dose of study medication was administered at the study site during visit 2 (baseline), and the remaining doses were dispensed for owner administration to the dog at home. Owners were provided a diary at visit 2 for recording study drug treatment, use of other medications, and safety observations. Between office-based visit 2 and visit 3, a telephone call occurred to interview the owner at day 8.

Dose selection

Doses selected were based on information available from preclinical studies, including efficacy and margin of safety (MOS) for the experimental drugs, and from the Food and Drug Administration–approved label for carprofen (*49*). The selected dose of LYA was based on the predicted mean trough plasma concentration equal to the in vitro IC_{80} of a dog whole blood PGE_2 synthesis assay, and a sufficient MOS based on a 2-week dog toxicology study (*27*). The assay for mPGES1 drug discovery operations used human whole blood to measure the ability of a compound to inhibit production of PGE_2 after lipopolysaccharide stimulation. The geometric mean IC_{50} (×/÷ geometric SD factor) for this compound in human blood was 6.78 nM (1.99), based on 14 samples. A similar assay using canine whole blood showed IC_{50} (×/÷ geometric SD factor) of 23.2 nM (1.53), based on 12 samples. The target dose for LYA was 1.5 mg/kg per day, split in two daily doses (0.75 mg/kg twice a day). The target dose for LYB was 25 mg/kg per day, in two daily doses of 12.5 mg/kg, based on a predicted mean trough plasma concentration greater than the in vitro IC_{50} of dog whole blood TNF release assay, and a MOS of ≥4× based on a 1-month dog toxicology study (*31*, *50*). The assay for EP4 drug discovery operations used whole blood to measure TNFα release secondary to EP4 antagonism. The geometric mean IC_{50} (×/÷ geometric SD factor) in human blood was 126 nM (2.26), based on 20 samples, and that in canine blood was 544 nM (1.53), based on 3 samples.

The dose of LYB was as high as possible to obtain an efficacy signal while maintaining an acceptable level of safety with respect to toxicological findings. The carprofen (Rimadyl) dose of 4.4 mg/kg per day was split into two daily doses of 2.2 mg/kg. Dogs received the same number of capsules within each weight group (grouped in 4-kg increments from 15 to 50 kg) to maintain the blind, and all treatments were administered at about 12-hour intervals. The precise time of each dose and whether the full dose was actually consumed was captured in a diary provided to the owner at visit 2. Capsules could be given with or without food. The use of tramadol (dose at the investigator's discretion but <5 mg/kg per day) as rescue therapy was permitted if the owner believed that the dog was experiencing excessive pain.

Outcome measures

To minimize enrollment eligibility bias to the primary endpoint scale, the LOAD mobility scale was used for screening and randomization as well as a secondary endpoint measure. The CBPI was not used for screening and randomization, but its PIS subscale was the primary endpoint.

Owner assessment of pain associated with OA (CBPI)

The validated scale (*14*) comprising 10 questions in two domains and a single overall quality of life question was completed by the dog owner at visit 1, visit 2, visit 3 (primary endpoint), and visit 4. The PIS domain comprises six questions that assess how pain affects the dog's "general activity," "enjoyment of life," and ability to "walk," "run," "rise," and "climb." The PSS domain of the CBPI comprises four questions that assess the pain at its "least," "worst," "on average," and "right now." The response options are an 11-point numerical scale with verbal anchors at each extreme, and domain

pain interference and pain severity subscores are the arithmetic mean of the items in each domain. In addition, there is a single "overall impression" item with which the owners provide categorical assessment ("poor," "fair," "good," "very good," or "excellent") of the dog's overall quality of life. The time frame of reference for the assessment was "the past 7 days." By design, we expected that dogs with LOAD scores in the moderate range would have correspondingly moderate CBPI scores. However, to ensure against dogs with low pain levels at randomization (the baseline pain score) and against dogs with highly variable scores across time (making them poor candidates for this short study), the protocol required that the CBPI should not have decreased by more than three points between screening and randomization.

Owner assessment of mobility in OA

The LOAD is a validated scale (*51*) completed by the owner in which mobility is assessed over the past 7 days through the owner's responses to a 13-item questionnaire in which each question has five categorical responses. Each item is scored 0 to 4, and the item scores are summed to give an overall instrument score, ranging from 0 to 52. This study targeted dogs with moderate pain, and thus, the inclusion criterion for LOAD mobility overall score at screening and randomization was ≥13 and ≤46. LOAD mobility was also assessed as a secondary efficacy endpoint.

Pharmacokinetics

The PK samples of LYA and LYB were collected at 1 and 3 hours after the first dose on day 1, as well as within 30 min before the day 15 dose, and 1 and ≥3 hours after the day 15 dose. Given the offsite location of study participants, the sampling schedule considered the practical duration for which the owners could stay at the clinic. With conventional noncompartmental analysis methods, limited PK parameters can be obtained, which include the observed maximum concentration (C_{max}), observed trough concentration (C_{trough}), and observed time of C_{max} (t_{max}) at steady state. Using a population PK analysis method, which uses the totality of the PK data from all animals without requiring intensive sampling in any single patient, more PK parameters can be estimated (*52*). In this study, the PK of both drugs was also analyzed with population PK analysis methods based on a one-compartment model with first-order absorption to obtain PK parameters such as apparent clearance (CL/F), apparent volume of distribution (V/F), half-life ($t_{1/2}$), and steady-state area under the concentration versus time curve over 24 hours ($AUC_{ss,24hr}$).

Safety

Blood was collected from each dog during each visit to monitor safety. Summaries were prepared for all TEAEs occurring during the treatment period, SAEs occurring on or after visit 2 (whether treatment-emergent or not), and AEs resulting in discontinuation from the study. The incidence rates of patients with treatment-emergent abnormal, high, or low laboratory values based on central laboratory reference ranges during the treatment period were also assessed.

Statistical analysis

All efficacy and safety analyses were conducted on the full analysis set, which included all data from all randomized dogs receiving at least one dose of the investigational product. All statistical comparisons were performed using an α of 5% with no adjustments for multiplicity. Efficacy measurements from the CBPI and LOAD mobility were analyzed using Bayesian mixed-effect model for repeated measures (MMRMs), with treatment, time (2- or 4-week assessment), and interaction of treatment and time as fixed effects and with baseline score, site, and weight as covariates. Time was fitted as a repeated effect within each dog, and an unstructured variance-covariance matrix was used. Posterior means and 95% credible intervals in the correct term for changes from baseline were reported for each treatment group, and the posterior probability that the pairwise difference in the change from baseline relative to placebo was less than 0 for each active dose arm was also calculated. The proportion of dogs meeting CBPI responder criteria at week 2 was analyzed and compared among treatment groups via logistic regression with covariates of site, weight, and baseline score in the model. The number of dogs with AEs was summarized, and pairwise comparisons relative to placebo were performed using Fisher's exact test. Similarly, pairwise comparisons with placebo for demographic variables were performed using *t* tests for continuous variables and Fisher's exact test for categorical variables.

SUPPLEMENTARY MATERIALS

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Table S1. Proportion of CBPI PIS responders after 2 weeks of treatment.

Table S2. Rates of treatment-emergent abnormal laboratory values occurring in ≥5% of dogs in any treatment group during the treatment period.

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. A. S. Lee, M. B. Ellman, D. Yan, J. S. Kroin, B. J. Cole, A. J. van Wijnen, H. J. Im, A current review of molecular mechanisms regarding osteoarthritis and pain. *Gene* **527**, 440–447 (2013).
2. N. Arden, M. C. Nevitt, Osteoarthritis: Epidemiology. *Best Pract. Res. Clin. Rheumatol.* **20**, 3–25 (2006).
3. D. J. Hunter, J. J. McDougall, F. J. Keefe, The symptoms of OA and the genesis of pain. *Rheum. Dis. Clin. North Am.* **34**, 623–643 (2008).
4. S. A. Martinez, Congenital conditions that lead to osteoarthritis in the dog. *Vet. Clin. North Am. Small Anim. Pract.* **27**, 735–758 (1997).
5. S. A. Martinez, G. S. Coronado, Acquired conditions that lead to osteoarthritis in the dog. *Vet. Clin. North Am. Small Anim. Pract.* **27**, 759–775 (1997).
6. T. Neogi, The epidemiology and impact of pain in osteoarthritis. *Osteoarthr. Cartil.* **21**, 1145–1153 (2013).
7. P. Rialland, S. Bichot, M. Moreau, M. Guillot, B. Lussier, D. Gauvin, J. Martel-Pelletier, J. P. Pelletier, E. Troncy, Clinical validity of outcome pain measures in naturally occurring canine osteoarthritis. *BMC Vet. Res.* **8**, 162 (2012).
8. O. G. Berge, Predictive validity of behavioural animal models for chronic pain. *Br. J. Pharmacol.* **164**, 1195–1206 (2011).
9. P. McGonigle, B. Ruggeri, Animal models of human disease: Challenges in enabling translation. *Biochem. Pharmacol.* **87**, 162–171 (2014).
10. M. H. Gregory, N. Capito, K. Kuroki, A. M. Stoker, J. L. Cook, S. L. Sherman, A review of translational animal models for knee osteoarthritis. *Arthritis* **2012**, 764621 (2012).
11. D. Knazovicky, E. S. Helgeson, B. Case, M. E. Gruen, W. Maixner, B. D. Lascelles, Widespread somatosensory sensitivity in naturally occurring canine model of osteoarthritis. *Pain* **157**, 1325–1332 (2016).
12. B. D. X. Lascelles, D. C. Brown, W. Maixner, J. S. Mogil, Spontaneous painful disease in companion animals can facilitate the development of chronic pain therapies for humans. *Osteoarthritis Cartilage* **26**, 175–183 (2018).
13. O. Vainio, Translational animal models using veterinary patients—An example of canine osteoarthritis (OA). *Scand. J. Pain* **3**, 84–89 (2012).
14. D. C. Brown, R. C. Boston, J. C. Coyne, J. T. Farrar, Ability of the Canine Brief Pain Inventory to detect response to treatment in dogs with osteoarthritis. *J. Am. Vet. Med. Assoc.* **233**, 1278–1283 (2008).
15. D. C. Brown, M. Bell, L. Rhodes, Power of treatment success definitions when the Canine Brief Pain Inventory is used to evaluate carprofen treatment for the control of pain and inflammation in dogs with osteoarthritis. *Am. J. Vet. Res.* **74**, 1467–1473 (2013).
16. H. Kapstad, B. Rokne, K. Stavem, Psychometric properties of the Brief Pain Inventory among patients with osteoarthritis undergoing total hip replacement surgery. *Health Qual. Life Outcomes* **8**, 148 (2010).

17. A. Kol, B. Arzi, K. A. Athanasiou, D. L. Farmer, J. A. Nolta, R. B. Rebhun, X. Chen, L. G. Griffiths, F. J. Verstraete, C. J. Murphy, D. L. Borjesson, Companion animals: Translational scientist's new best friends. *Sci. Transl. Med.* **7**, 308ps321 (2015).
18. J. Sokolove, C. M. Lepus, Role of inflammation in the pathogenesis of osteoarthritis: Latest findings and interpretations. *Ther. Adv. Musculoskelet. Dis.* **5**, 77–94 (2013).
19. S. A. Johnston, S. M. Fox, Mechanisms of action of anti-inflammatory medications used for the treatment of osteoarthritis. *J. Am. Vet. Med. Assoc.* **210**, 1486–1492 (1997).
20. B. P. Monteiro-Steagall, P. V. Steagall, B. D. Lascelles, Systematic review of nonsteroidal anti-inflammatory drug-induced adverse effects in dogs. *J. Vet. Intern. Med.* **27**, 1011–1019 (2013).
21. S. Harirforoosh, W. Asghar, F. Jamali, Adverse effects of nonsteroidal antiinflammatory drugs: An update of gastrointestinal, cardiovascular and renal complications. *J. Pharm. Pharm. Sci.* **16**, 821–847 (2013).
22. B. J. R. Whittle, Gastrointestinal effects of nonsteroidal anti-inflammatory drugs. *Fundam. Clin. Pharmacol.* **17**, 301–313 (2003).
23. I. A. Tavares, A. Bennett, Non-steroidal anti-inflammatory drugs that cause relatively little gastric damage. *J. Gastroenterol. Hepatol.* **13**, S190–S192 (1998).
24. S. Tacconelli, A. Bruno, R. Grande, P. Ballerini, P. Patrignani, Nonsteroidal anti-inflammatory drugs and cardiovascular safety—Translating pharmacological data into clinical readouts. *Expert Opin. Drug Saf.* **16**, 791–807 (2017).
25. B. B. Weksler, Prostanoids and NSAIDs in cardiovascular biology and disease. *Curr. Atheroscler. Rep.* **17**, 41 (2015).
26. A. Dray, S. J. Read, Arthritis and pain. Future targets to control osteoarthritis pain. *Arthritis Res. Ther.* **9**, 212 (2007).
27. S. Chandrasekhar, A. K. Harvey, X. P. Yu, M. G. Chambers, J. L. Oskins, C. Lin, T. W. Seng, S. J. Thibodeaux, B. H. Norman, N. E. Hughes, M. A. Schiffler, M. J. Fisher, Identification and characterization of novel microsomal prostaglandin E synthase-1 inhibitors for analgesia. *J. Pharmacol. Exp. Ther.* **356**, 635–644 (2016).
28. M. Korotkova, P. J. Jakobsson, Microsomal prostaglandin e synthase-1 in rheumatic diseases. *Front. Pharmacol.* **1**, 146 (2011).
29. A. Koeberle, S. A. Laufer, O. Werz, Design and development of microsomal prostaglandin E2 synthase-1 inhibitors: Challenges and future directions. *J. Med. Chem.* **59**, 5970–5986 (2016).
30. S. L. Kuklish, S. Antonysamy, S. N. Bhattachar, S. Chandrasekhar, M. J. Fisher, A. J. Fretland, K. Gooding, A. Harvey, N. E. Hughes, J. G. Luz, P. R. Manninen, J. E. McGee, A. Navarro, B. H. Norman, K. M. Partridge, S. J. Quimby, M. A. Schiffler, A. V. Sloan, A. M. Warshawsky, J. S. York, X. P. Yu, Characterization of 3,3-dimethyl substituted *N*-aryl piperidines as potent microsomal prostaglandin E synthase-1 inhibitors. *Bioorg. Med. Chem. Lett.* **26**, 4824–4828 (2016).
31. S. Chandrasekhar, X. P. Yu, A. K. Harvey, J. L. Oskins, C. Lin, X. Wang, M. J. Blanco, M. J. Fisher, S. L. Kuklish, M. A. Schiffler, T. Vetman, A. M. Warshawsky, J. S. York, A. M. Bendele, M. G. Chambers, Analgesic and anti-inflammatory properties of novel, selective, and potent EP4 receptor antagonists. *Pharmacol. Res. Perspect.* **5**, e00316 (2017).
32. U.S. Food and Drug Administration, Galliprant—A nonsteroidal anti-inflammatory drug (NSAID) for dogs with osteoarthritis (U.S. Food and Drug Administration, 2018); www.fda.gov/animal-veterinary/animal-health-literacy/galliprant-nonsteroidal-anti-inflammatory-drug-nsaid-dogs-osteoarthritis [accessed 16 July 2019].
33. K. Kirkby Shaw, L. C. Rausch-Derra, L. Rhodes, Grapiprant: An EP4 prostaglandin receptor antagonist and novel therapy for pain and inflammation. *Vet. Med. Sci.* **2**, 3–9 (2016).
34. L. Rausch-Derra, M. Huebner, J. Wofford, L. Rhodes, A prospective, randomized, masked, placebo-controlled multisite clinical study of grapiprant, an EP4 prostaglandin receptor antagonist (PRA), in dogs with osteoarthritis. *J. Vet. Intern. Med.* **30**, 756–763 (2016).
35. M. J. Blanco, T. Vetman, S. Chandrasekhar, M. J. Fisher, A. Harvey, S. L. Kuklish, M. Chambers, C. Lin, D. Mudra, J. Oskins, X. S. Wang, X. P. Yu, A. M. Warshawsky, Identification and biological activity of 6-alkyl-substituted 3-methyl-pyridine-2-carbonyl amino dimethyl-benzoic acid EP4 antagonists. *Bioorg. Med. Chem. Lett.* **26**, 2303–2307 (2016).
36. Y. Jin, C. Smith, L. Hu, D. E. Coutant, K. Whitehurst, K. Phipps, T. A. McNearney, X. Yang, B. Ackermann, T. Pottanat, W. Landschulz, LY3127760, a selective prostaglandin E4 (EP4) receptor antagonist, and celecoxib: A comparison of pharmacological profiles. *Clin. Transl. Sci.* **11**, 46–53 (2018).
37. M. Payne-Johnson, C. Becskei, Y. Chaudhry, M. R. Stegemann, Comparative efficacy and safety of mavacoxib and carprofen in the treatment of canine osteoarthritis. *Vet. Rec.* **176**, 284 (2015).
38. K. Ding, Z. Zhou, S. Hou, Y. Yuan, S. Zhou, X. Zheng, J. Chen, C. Loftin, F. Zheng, C.-G. Zhan, Structure-based discovery of mPGES-1 inhibitors suitable for preclinical testing in wild-type mice as a new generation of anti-inflammatory drugs. *Sci. Rep.* **8**, 5205 (2018).
39. B. KuKanich, T. Bidgood, O. Knesl, Clinical pharmacology of nonsteroidal anti-inflammatory drugs in dogs. *Vet. Anaesth. Analg.* **39**, 69–90 (2012).
40. P. K. Owens, E. Raddad, J. W. Miller, J. R. Stille, K. G. Olovich, N. V. Smith, R. S. Jones, J. C. Scherer, A decade of innovation in pharmaceutical R&D: The Chorus model. *Nat. Rev. Drug Discov.* **14**, 17–28 (2015).
41. D. C. Brown, R. C. Boston, J. T. Farrar, Comparison of force plate gait analysis and owner assessment of pain using the Canine Brief Pain Inventory in dogs with osteoarthritis. *J. Vet. Intern. Med.* **27**, 22–30 (2013).
42. M. G. Conzemius, R. B. Evans, Caregiver placebo effect for dogs with lameness from osteoarthritis. *J. Am. Vet. Med. Assoc.* **241**, 1314–1319 (2012).
43. A. Hróbjartsson, P. C. Gøtzsche, Is the placebo powerless? *N. Engl. J. Med.* **344**, 1594–1602 (2001).
44. S. Malek, S. J. Sample, Z. Schwartz, B. Nemke, P. B. Jacobson, E. M. Cozzi, S. L. Schaefer, J. A. Bleedorn, G. Holzman, P. Muir, Effect of analgesic therapy on clinical outcome measures in a randomized controlled trial using client-owned dogs with hip osteoarthritis. *BMC Vet. Res.* **8**, 185 (2012).
45. A. Gagnon, D. Brown, M. Moreau, B. Lussier, C. Otis, E. Troncy, Therapeutic response analysis in dogs with naturally occurring osteoarthritis. *Vet. Anaesth. Analg.* **44**, 1373–1381 (2017).
46. M. Moreau, P. Rialland, J. P. Pelletier, J. Martel-Pelletier, D. Lajeunesse, C. Boileau, J. Caron, D. Frank, B. Lussier, J. R. del Castillo, G. Beauchamp, D. Gauvin, T. Bertaim, D. Thibaud, E. Troncy, Tiludronate treatment improves structural changes and symptoms of osteoarthritis in the canine anterior cruciate ligament model. *Arthritis Res. Ther.* **13**, R98 (2011).
47. M. P. Klinck, J. S. Mogil, M. Moreau, B. D. X. Lascelles, P. A. Flecknell, T. Poitte, E. Troncy, Translational pain assessment: Could natural animal models be the missing link? *Pain* **158**, 1633–1646 (2017).
48. Veterinary International Conference on Harmonization, Good Clinical Practices: VICH GL9 (GCP) (2001); www.vichsec.org/guidelines/pharmaceuticals/pharma-efficacy/good-clinical-practice.html [accessed 8 September 2017].
49. U.S. Food and Drug Administration, RIMADYL (Carprofen, USP) (2012); www.fda.gov/downloads/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/DrugLabels/UCM050406.pdf [accessed 24 May 2017].
50. A. Murase, Y. Taniguchi, H. Tonai-Kachi, K. Nakao, J. Takada, In vitro pharmacological characterization of CJ-042794, a novel, potent, and selective prostaglandin EP(4) receptor antagonist. *Life Sci.* **82**, 226–232 (2008).
51. M. B. Walton, E. Cowderoy, D. Lascelles, J. F. Innes, Evaluation of construct and criterion validity for the 'Liverpool Osteoarthritis in Dogs' (LOAD) clinical metrology instrument and comparison to two other instruments. *PLoS One* **8**, e58125 (2013).
52. E. Samara, R. Granneman, Role of population pharmacokinetics in drug development. A pharmaceutical industry perspective. *Clin. Pharmacokinet.* **32**, 294–312 (1997).

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A randomized clinical efficacy study targeting mPGES1 or EP4 in dogs with spontaneous osteoarthritis

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Paws on pain

Canines experience osteoarthritis similar to humans, and studies testing therapeutics in companion animals can provide valuable support for clinical trials. Robertson-Plouch and colleagues evaluated efficacy of inhibitors of the prostaglandin E (PGE) pathway in reducing pain in dogs with spontaneously occurring osteoarthritis. A microsomal PGE synthase-1 inhibitor showed promising results for reducing pain scores compared to a nonsteroidal anti-inflammatory targeting cyclooxygenase-2 and a downstream antagonist of the E-type prostanoid receptor 4, although consistent superiority compared to placebo did not reach the study's predetermined threshold. Results from this proof-of-concept study support further investigation of microsomal PGE synthase-1 inhibition for analgesia.