JAVMA

Delayed-release rapamycin halts progression of left ventricular hypertrophy in subclinical feline hypertrophic cardiomyopathy: results of the RAPACAT trial

Joanna L. Kaplan, DVM, DACVIM[1]; Victor N. Rivas, MS[1,2]; Ashley L. Walker, DVM[1]; Louise Grubb, BSc, MBS[3]; Aisling Farrell, MPSI, MSc, PGCert[3]; Stuart Fitzgerald, MVB, MANZCVS[3]; Susan Kennedy, BSc, PhD[3]; Carina E. Jauregui, RVT, RLAT[1,2]; Amanda E. Crofton, DVM[1]; Chris McLaughlin, DVM, DACVECC[2]; Rachel Van Zile, DVM[2]; Teresa C. DeFrancesco, DVM, DACVECC, DACVIM[2]; Kathryn M. Meurs, DVM, PhD, DACVIM[2]; Joshua A. Stern, DVM, PhD, DACVIM[1,2]*

[1]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA
[2]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC
[3]TriviumVet, Waterford, Ireland

*Corresponding author: Dr. Stern (jastern@ncsu.edu)

Received April 7, 2023
Accepted June 27, 2023

doi.org/10.2460/javma.23.04.0187

OBJECTIVE
Feline hypertrophic cardiomyopathy (HCM) remains a disease with little therapeutic advancement. Rapamycin modulates the mTOR pathway, preventing and reversing cardiac hypertrophy in rodent disease models. Its use in human renal allograft patients is associated with reduced cardiac wall thickness. We sought to evaluate the effects of once-weekly delayed-release (DR) rapamycin over 6 months on echocardiographic, biochemical, and biomarker responses in cats with subclinical, nonobstructive HCM.

ANIMALS
43 client-owned cats with subclinical HCM.

METHODS
Cats enrolled in this double-blinded, multicentered, randomized, and placebo-controlled clinical trial were allocated to low- or high-dose DR rapamycin or placebo. Cats underwent physical examination, quality-of-life assessment, blood pressure, hematology, biochemistry, total T4, urinalysis, N-terminal pro-B-type natriuretic peptide, and cardiac troponin I at baseline and days 60, 120, and 180. Fructosamine was analyzed at screening and day 180. Echocardiograms were performed at all time points excluding day 120. Outcome variables were compared using a repeated measures ANCOVA.

RESULTS
No demographic, echocardiographic, or clinicopathologic values were significantly different between study groups at baseline, confirming successful randomization. At day 180, the primary study outcome variable, maximum LV myocardial wall thickness at any location, was significantly lower in the low-dose DR rapamycin group compared to placebo ($P = .01$). Oral DR rapamycin was well tolerated with no significant differences in adverse events between groups.

CLINICAL RELEVANCE
Results demonstrate that DR rapamycin was well tolerated and may prevent or delay progressive LV hypertrophy in cats with subclinical HCM. Additional studies are warranted to confirm and further characterize these results.

Keywords: HCM, sirolimus, occult, cat, mTOR

Hypertrophic cardiomyopathy (HCM) is the most common cardiovascular disease in domestic cats with a prevalence of 14%.[1–3] HCM is defined as asymmetric or symmetric thickening of the left ventricular posterior wall (LVPW) or interventricular septum (IVS) in the absence of any hemodynamic cause (ie, systemic hypertension, congenital disease, or fixed stenosis) or hormonal stimuli (ie, hyperthyroidism or hypersomatotropism).[1,2] The disease is typically irreversible and progressive. While some cats remain subclinical throughout life, many progress to develop 1 or a combination of severe outcomes including left-sided congestive heart failure (CHF), arterial thromboembolism (ATE), and sudden death with reported

occurrence rates of 23.9%, 11.3%, and 3.4%, respectively.[4] Despite extensive investigative efforts, current available drug therapies have failed to delay progression of disease, improve quality of life (QOL), or show survival benefit in the subclinical setting.[1,2] A contributing factor may be that many existing drug therapies target the hemodynamic consequences of HCM rather than abnormal molecular pathways or proteins involved in initiating the disease. Therefore, it is imperative to explore novel therapeutic targets to treat cats with occult HCM.

Rapamycin, also known as sirolimus, a macrolide and inhibitor of the mechanistic (previously called mammalian) target of rapamycin (mTOR), shows promise as a novel therapeutic for feline HCM.[5–10] mTOR is an atypical serine/threonine protein kinase that associates with several proteins to form 1 of 2 multiprotein complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2).[5,11,12] These 2 complexes have distinct roles in promoting cardiovascular health during both embryogenesis and postnatal life with varying up- and downstream targets. Activation of mTORC1 promotes anabolic processes that increase both protein and lipid synthesis, downregulates catabolic processes to reduce autophagy (the cell's ability to remove damaged proteins and organelles), and plays a vital role in signaling adaptive cardiac remodeling in response to mechanical overload.[5] Although not completely understood, mTORC2 plays a role in regulating glucose and lipid metabolism and promotes cardiomyocyte cell survival, cytoskeletal organization, and appropriate cell polarity.

Both mTORC1 and mTORC2 are essential for normal cardiovascular health; complete disruption of these complexes leads to metabolic derangements and cardiovascular disease.[5] However, studies have demonstrated that mTORC1 activation from chronic hemodynamic stressors may be detrimental to the heart, leading to pathologic hypertrophy. While mTORC1 is acutely sensitive to rapamycin, the mTORC2 protein complex is comparatively insensitive. Prolonged chronic exposure to rapamycin is required to disrupt mTORC2 in vivo or in cell culture.[12] Inhibition of mTORC2 has been shown to result in glucose intolerance and hepatic insulin resistance.[13] Intermittent rapamycin dosing has been shown to mitigate the deleterious effects of prolonged mTORC2 inhibition while enabling sustained inhibition of mTORC1 in many tissues.[14] Numerous studies involving mouse models of HCM, as well as a human study in renal transplant patients, have demonstrated that inhibition of mTORC1 with rapamycin resulted in reversed pathologic left ventricular (LV) hypertrophy.[6–10] Additionally, a study[15] of dogs demonstrated beneficial effects of intermittent dosing of rapamycin in aging hearts, including improved diastolic and systolic indices of heart function.

Once-weekly administration of a new formulation of delayed-released (DR) rapamycin has recently been developed for treatment of feline HCM. The objective of this study was to evaluate the safety and efficacy in reversing or delaying progression of myocardial hypertrophy and consequently cardiac dysfunction via once-weekly oral dosing of DR rapamycin in client-owned cats with subclinical HCM over a 6-month period. We hypothesized that once-weekly dosing of rapamycin would be well tolerated in cats and either reverse or prevent progression of LV remodeling echocardiographically.

Methods

Animals

The study was conducted in accordance with protocol FDA 21 CFR Part 511.1(b), *New Animal Drugs for Clinical Investigation in Animals*, and FDA 21 CFR Part 11, *Electronic Records, Electronic Signatures*. All study procedures were approved by the IACUC at the University of California-Davis School of Veterinary Medicine (protocol No. 21895) and North Carolina State University College of Veterinary Medicine (protocol No. 20-342-O). All clients gave informed signed consent prior to enrollment.

Client-owned cats < 12 years of age, ≥ 3.3 kg, and with an echocardiographic diagnosis of subclinical HCM were recruited to either of the 2 enrollment centers for the study. A diagnosis of HCM was made if there was echocardiographic evidence of symmetric or asymmetric LV wall thickening defined as an LV wall thickness of ≥ 6 mm at end diastole by 2-D or M-mode assessment. Careful attention was taken to exclude insertion sites of false tendons.[2]

Study design

A summary of the study design is provided **(Table 1)**. This study was a randomized, double-blinded, placebo-controlled clinical trial involving 2 tertiary referral centers. At time of recruitment (days −14 to −1), a full medical history was obtained and all cats underwent a full physical exam, echocardiogram, 3-minute 6-lead ECG, 3-view thoracic radiographs, a Doppler systolic blood pressure, hematology, biochemistry, serum total T4, serum fructosamine, plasma N-terminal pro-B-type natriuretic peptide (NTproBNP), serum cardiac troponin I (CTnI), and urinalysis (urine obtained via ultrasound-guided cystocentesis). Owners were instructed to fill out the Cats' Assessment Tool for Cardiac Health questionnaire for QOL assessments.[16]

Cats meeting all inclusion criteria and no exclusion criteria were enrolled in the study at least 1 day and no more than 14 days after the initial screening visit. At time of enrollment (considered day 0), cats were randomized by a treatment dispenser independent of any data collection to 1 of 3 groups: placebo, DR rapamycin 0.3 mg/kg (low-dose), or DR rapamycin 0.6 mg/kg (high-dose) in a 1:1:1 ratio. The formulation of rapamycin used in this study is currently not commercially available for veterinary use and is restricted to ongoing clinical trials while conditional approval is sought from the FDA Center for Veterinary Medicine. These doses were supported by a toxicokinetic study[17] conducted by the sponsor that demonstrated the safety of repeated dosing with DR rapamycin in cats within this range. Randomization was performed using Java cryptographically strong

Table 1—Clinical trial schedule and procedural specifications.

Procedure	Screening days −14 to −1	Enrollment day 0	Day 60 ± 10	Day 120 ± 10	Day 180 ± 10
Informed consent	X				
Medical history	X				
Physical examination	X		X	X	X
Quality of life assessment	X		X	X	X
Echocardiography	X		X		X
3-min electrocardiogram	X				
Thoracic radiographs	X				
Systolic blood pressure	X		X	X	X
Hematology	X		X	X	X
Biochemistry	X		X	X	X
Total T4	X		X	X	X
Fructosamine	X				X
Urinalysis	X		X	X	X
NTproBNP	X		X	X	X
Cardiac troponin I	X		X	X	X
IVP/placebo dispensing		X	X	X	
IVP/placebo accountability			X	X	X

IVP = Investigational veterinary product. NTproBNP = N-terminal pro-B-type natriuretic peptide.

SecureRandom number generator through VISION software (version 10.0; Prelude Dynamics).

Exclusion criteria included evidence of severe LV outflow tract obstruction (LVOTO) defined as an LVOT maximum pressure gradient > 50 mm Hg, any additional congenital or acquired cardiac disease identified on echocardiogram other than HCM, systemic hypertension (systolic blood pressure [BP] ≥ 170 mm Hg), hyperthyroidism, diabetes mellitus (DM), renal dysfunction (defined as a creatinine > 2.1 mg/dL), moderate to severe anemia defined as an Hct or PCV < 25%, evidence of CHF (defined by a combination of dyspnea, evidence of pulmonary edema identified on thoracic radiographs, cavitary effusion identified on echocardiogram or thoracic radiographs, or other signs attributed by the investigator to be consistent with CHF), current or previous ATEs, or cardiac arrhythmias considered hemodynamically significant by the investigator that warranted antiarrhythmic therapy. Cats were excluded if oral medication administration was unsuccessful or long-term corticosteroids were administered. Additional prohibited concomitant therapies included inhibitors or inducers of cytochrome P-450 3A4 and p-glycoprotein including but not restricted to the following: antifungal agents, β blocking agents, calcium channel blockers, cimetidine, cisapride, cyclosporine, digoxin, macrolide antibiotics, metoclopramide, phenobarbital, phenytoin, pimobendan, rifampin, sarcomeric inhibiting compounds, or any thiazide, loop, and/or potassium-sparing diuretics. Concomitant therapies that were permitted included nonsteroidal anti-inflammatory drugs or antibiotics if deemed necessary by the investigator, oral clopidogrel, and angiotensin-converting enzyme inhibitors at standard dosing. All permitted concomitant medications were required to be administered for at least 2 weeks at a stable dose prior to study enrollment; new cardiac medications were not permitted to be instituted during the course of the study.

Following enrollment, cats were required to return for follow-up visits on days 60, 120, and 180 (all ± 10 days). All follow-up visits included a full physical exam, Doppler BP, urinalysis, and blood collection for hematology, biochemistry, total T4, NTproBNP, and cTnI. A full echocardiogram was repeated on days 60 and 180. Owners were required to fill out the Cats' Assessment Tool for Cardiac Health questionnaire at each follow-up visit as well as an owner diary documenting all, if any, abnormal observations or perceived adverse events between study visits.[16]

Drug administration and owner compliance

Drug or placebo tablets were administered orally every 7 (± 2) days by the owner. If the cat vomited within 30 minutes of dosing, owners were instructed to give a new dose within a 12-hour time frame.

Owners were required to record all time points and days in which the drug was given on a treatment log provided by the study investigators. Owners were also instructed to return any unused product to the clinic at each follow-up visit. At each visit, the treatment log was reviewed; any unused drug was tabulated by the treatment dispenser to ensure adequate owner compliance. Owners were not permitted to miss > 25% of product administration and remain in the study.

Masking and study withdrawal

All study personnel, excluding the treatment dispensers, sponsor, and study monitor, were blinded to treatment. Unblinding could occur if necessary for the welfare of the cat. Owners maintained the option to remove a cat from the study at any time regardless of the reason. In addition, cats were removed from the study for any of the following reasons: if they were treated with a prohibited medication, had significant changes made to their diet, the subject or owner was not compliant with study procedures (ie, inability to dose on at least 75% of scheduled dosing days between visits or persistent noncompliance), there was a protocol violation, or a significant adverse event occurred, requiring the cat to be discontinued from the study treatment at the discretion of the study investigator.

Study patients were to be removed postenrollment if they were lost to follow-up; if there was development of pericardial effusion or ascites, significant cytopenias (Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events [VCOG-CTCAE] ≥ grade 3), or physical or biochemical (VCOG-CTCAE ≥ grade 3)[18] evidence of organ dysfunction; if a diagnosis of DM or hypertension (systolic BP ≥ 170 mm Hg) was made; and/or if the sponsor requested early termination of the study.

Sample collection and analysis

Blood and urine were collected via a cephalic or medial saphenous vein and ultrasound-guided cystocentesis, respectively. Blood and urine samples for hematology, biochemistry, total T4, NTproBNP, fructosamine, and urinalysis were submitted to IDEXX laboratories for analysis. Serum was stored at −80 °C and submitted in batch to the Texas A&M Gastrointestinal Laboratory for high-sensitivity (HS) and ultra-sensitive (US) cTnI analysis.

Echocardiography

Routine echocardiography was performed by a board-certified cardiologist or resident in training under the supervision of a board-certified cardiologist. All echocardiographic measurements and calculations were performed at a digital off-cart workstation (Syngo Dynamic Workplace; Siemens Medical Solutions; and Studycast; Core Sound Imaging). Maximal left atrial (LA) diameter was measured in a routine right parasternal 4-chamber long-axis view.[2,19,20] LVOT maximal velocity was performed using continuous wave Doppler in the LVOT in systole in the routine left apical 5-chamber view. In 2-D planes, diastolic IVS thickness (IVSd) was measured using a leading edge-to-trailing edge technique and diastolic LVPW (LVPWd) was measured using a leading edge-to-leading edge technique.[2] Care was taken to avoid insertion sites of moderator bands. All 2-D–guided M-mode measurements of IVSd and LVPWd were measured using a leading edge-to-leading edge technique. Measurements were taken as the average of 3 consecutive cardiac cycles when possible, avoiding any noted arrhythmias.

Data collection and analysis

All data were recorded electronically in real time through VISION software (version 10.0; Prelude Dynamics). Data input was monitored by Argenta Global, a contract research organization. To evaluate the efficacy of rapamycin low-dose and high-dose treatment, maximum wall thickness (MWT) change of the LV (of either IVSd or LVPWd as measured by M-mode or 2-D, whichever was greatest) was assessed as the primary end point. Maximum LA diameter, IVSd, and LVPWd were also evaluated independently from one another. Exploratory analyses of the relationship between disease progression and response, as well as baseline patient characteristics, were also conducted.

Statistical analysis

Sample size determination—A power analysis indicated that 36 cats (n = 12/group), at an α error = 0.1, and a detectible difference beyond biologic variability of 0.5 mm between baseline and day 180, would yield a β error of < 0.1 (statistical power of > 90%) to identify clinically relevant differences between baseline and post-treatment echocardiographic assessment of LV wall thickness in diastole. The model used for this calculation was a paired sampling difference between baseline and post-treatment echocardiographic variables and utilized a SD of 0.4 mm from the laboratories' unpublished data.

Efficacy analysis—Statistical analyses were carried out using commercially available software (SAS/STAT version 9.4; SAS Institute Inc; and Prism version 9.5.1; GraphPad Software). The parameters IVSd, LVPWd, and maximum LA diameter values were subject to a repeated-measures ANCOVA (RMANCOVA). Maximum wall thickness at each exam was used in the statistical analysis of IVSd and LVPWd. The statistical model included treatment, time, and the treatment by time interaction as fixed effects. The screening value was used as a covariate. A compound symmetric structure was assumed for the covariance matrix given only 2 measurement time points after the onset of treatment. Where the treatment by time interaction was significant, within time treatment effects were evaluated by comparing the 2 investigational veterinary product (IVP) groups to the control group and comparing the pooled IVP groups to the control group using linear contrasts. Where the interaction was not significant, the main effect of treatment was assessed, and where significant, the 2 IVP groups were compared to the control group, and the pooled IVP groups were compared to the control group using a linear contrast. Where the treatment by day interaction was not significant, the 2 time points (day 60 and day 180) were pooled (pooled across days) and compared between each treatment and the control group. Due to the nonpivotal, exploratory, and dose-determining nature of this study, contrasts were evaluated using an $\alpha_{unadjusted} = 0.10$. Study site was not considered in the statistical analysis. Data were permitted to be appropriately transformed prior to statistical analysis if the assumptions of a normal distribution were not met; however, data from these outcomes followed a normal distribution and thus no transformations were needed. The percent of animals with a change from screening > 0.5 mm was calculated for both time points and subject to statistical analysis using a Fisher's exact test at each time point **(Supplementary Table S1)**.

In addition to the above analysis, the relationship between baseline values of MWT, NTproBNP, cTNI, and progression/response was evaluated in an effort to determine whether these parameters at baseline may be useful in predicting treatment outcome. Efficacy outcomes were evaluated at an α = 0.10 using 2-sided tests.

Safety analysis—Hematology and biochemistry results were subject to RMANCOVA, using the pretreatment value as a covariate. Treatment group, time, and time by treatment group interaction were included in the statistical model as fixed effects, and site, site by treatment, and

site by treatment by time interactions were included as random effects. Given only 3 time points post-treatment for the clinical pathology outcomes, a compound symmetric structure was assumed for the covariance matrix. Where the interaction was statistically significant, within-time differences between treatment groups were evaluated using an $\alpha_{unadjusted}$ = 0.10. Where the interaction was not significant, the main effect of treatment was assessed. Least-squares means and SEs were used to summarize the findings. Safety outcomes were evaluated at an α = 0.10 using 2-sided tests.

Results

A total of 53 cats were screened for the study. Doses administered to the 24 evaluable rapamycin-treated cats in the low-dose and high-dose groups were 0.25 to 0.38 mg/kg and 0.52 to 0.73 mg/kg, respectively. Of the cats recruited for screening, 9 were immediately excluded due to severe LVOTO (n = 4), negative or equivocal HCM status (3), body weight below inclusion criteria (1), and echocardiographic evidence of a ventricular septal defect (1). One cat was excluded from analysis after completing the study due to persistent hypercalcemia, present prior to enrollment, that was initially overlooked. A total of 43 cats were successfully enrolled. A total of 36 cats completed all follow-up visits until day 180, 38 cats completed visits up to day 120, and 41 cats completed visits up to day 60. The remaining 7 of 43 cats were excluded for the following reasons: initiation of clopidogrel during the study period (n = 1), loss of follow-up (1), owner request for withdrawal due to personal reasons unrelated to the study (1), development of left-sided CHF (1), development of left-sided CHF that resulted in humane euthanasia (1), development of seizures and death (1), and development of diabetic ketoacidosis (DKA) and death (1). The cat removed from the study due to starting clopidogrel was in the placebo group, the cat hospitalized for DKA was receiving the low-dose study drug, and all other cats withdrawn from the study were receiving the high-dose study drug.

Five cats experienced serious adverse events during the study period, 4 of which were removed from the study for reasons listed above. One serious adverse event was considered unrelated to treatment (development of a urethral obstruction), and the study subject remained in the study. Four of the cats with serious adverse events were in groups receiving rapamycin (2 cats progressed to CHF, 1 cat died suddenly, and 1 cat developed DKA) and 1 was in the placebo group; there were no difference between number of adverse events in the placebo versus treatment group (P > .99). Sixty-nine adverse events were observed during the study. Two adverse events were determined to be "probably" related to the IVP. Fourteen adverse events were determined to be "possibly" related to the IVP. The remaining 53 adverse events were determined to have an "unrelated" or "unknown" relationship to the IVP. A total of 21, 23, and 25 adverse events were observed for placebo, low-dose, and high-dose groups, respectively. No statistically significant difference between the number of adverse events between groups was noted (P = .82). A complete list of all adverse events is provided elsewhere **(Supplementary Table S2)**.

Of the 43 cats (6 spayed females and 37 castrated males) enrolled in the study at baseline, mean age was 6 years (range, 1 to 12 years) and was similar across groups. Breed and sex distribution among treatment groups is provided elsewhere **(Supplementary Table S3)**. Mean body weights for cats in the placebo, low-dose, and high-dose groups were 6.06 kg (range, 4.2 to 14 kg), 5.97 kg (range, 4 to 8.9 kg), and 4.94 kg (range, 3.3 to 6.3 kg), respectively. Mean body weight, respiratory rate, and heart rate were not significantly different among groups over time. No differences were observed in QOL scores, systolic BP, biochemistry or hematology values, total T4, or fructosamine between groups at baseline or at any additional time point during the study period. Proteinuria was detected in select cases in placebo, low-dose, and high-dose groups, but there was no treatment effect observed **(Supplementary Tables S4–S7)**.

Echocardiographic values were not significantly different between the 3 study groups at baseline. Following the 180-day treatment period, significant differences in LV MWT were identified **(Figure 1)**.

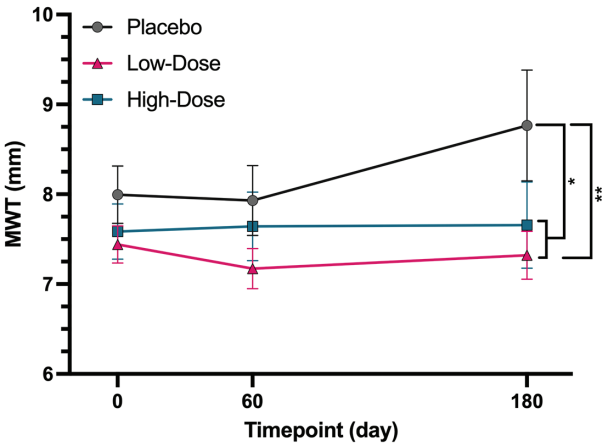

Figure 1—Temporal comparison of maximum wall thickness (MWT; mm) at any location between placebo (gray) and low- (pink) and high-dose (teal) delayed-release rapamycin. The * and ** denote a P value of .05 for pooled dose groups compared to placebo and .01 for low dose compared to placebo, respectively. Values are reported as mean ± SEM.

Following RMANCOVA of the primary efficacy outcome variables, a significant treatment by visit effect on MWT at any location was detected. Low-dose MWT values were significantly lower than values reported in the placebo group at visit 3 (day 180; P = .01). The pooled (low- and high-dose) IVP versus placebo comparison at visit 3 (day 180) also indicated a significant difference in MWT (P = .05). Additionally, a significant treatment effect on maximum LVPWd 2-D obtained from the right parasternal imaging window was detected. A summary of the RMANCOVA statistical analysis of the efficacy outcome variables is presented **(Tables 2 and 3)**.

Table 2—Summary of repeated-measures ANCOVA (RMANCOVA) statistical analysis on the efficacy outcome variables pooled across study sites.

Variable	Time point	Treatment group	N	LS mean	SEM	*P* values	*P* value
Maximum LA diameter (2-D)	Pooled across days	Placebo	25	15.63	0.39	Treatment * visit: .386	NA†
		Low dose	29	15.41	0.36		NA
		High dose	23	16.40	0.40		NA
	Day 60	Placebo	13	15.45	0.49	Treatment: .182	NA
		Low dose	15	15.77	0.45		NA
		High dose	13	16.29	0.49		NA
	Day 180	Placebo	12	15.81	0.51		NA
		Low dose	14	15.05	0.47		NA
		High dose	10	16.51	0.55		NA
Maximum LVPWd (maximum of M-mode and 2-D)	Pooled across days	Placebo	25	7.78	0.27	Treatment * visit: .162	NA
		Low dose	29	7.14	0.25		NA
		High dose	23	7.32	0.27		NA
	Day 60	Placebo	13	7.42	0.30	Treatment: .225	NA
		Low dose	15	7.14	0.28		NA
		High dose	13	7.19	0.30		NA
	Day 180	Placebo	12	8.15	0.31		NA
		Low dose	14	7.14	0.29		NA
		High dose	10	7.45	0.32		NA
Maximum IVSd (maximum of M-mode and 2-D)	Pooled across days	Placebo	25	6.70	0.18	Treatment * visit: .931	NA
		Low dose	29	6.83	0.17		NA
		High dose	23	6.98	0.19		NA
	Day 60	Placebo	13	6.64	0.23	Treatment: .555	NA
		Low dose	15	6.76	0.22		NA
		High dose	13	6.99	0.23		NA
	Day 180	Placebo	12	6.77	0.24		NA
		Low dose	14	6.89	0.22		NA
		High dose	10	6.97	0.27		NA
MWT at any location (n = 56 via maximum of LVPWd and n = 61 via IVSd M-mode and 2-D; 2 measures were the same for LVPWd and IVSd)	Pooled across days	Placebo	25	7.96	0.24	**Treatment * visit: .084**	—
		Low dose	29	7.36	0.22		NA‡
		High dose	23	7.87	0.25	Treatment: .150	NA
	Day 60	Placebo	13	7.52	0.27		—
		Low dose	15	7.30	0.25		.564
		High dose	13	7.72	0.27		.602
		IVP–pooled	28	7.51	—		.982
	Day 180	Placebo	12	8.40	0.28		—
		Low dose	**14**	**7.41***	**0.25**		**.013**
		High dose	10	8.02	0.29		.36
		IVP–pooled	**24**	**7.72***	**—**		**.054**

Statistically significant ($P < .1$) values are bolded.
IVSd = Diastolic interventricular septum. LA = Left atrial. LS mean = Least-squares mean. LVPWd = Diastolic left ventricular posterior wall. MWT = Maximum wall thickness.
†Not applicable. Neither the treatment by visit interaction nor the treatment effect were significant; no comparisons to the placebo were made. ‡Not applicable. The treatment by visit interaction was significant; within-visit comparisons were made.
See Table 1 for remainder of key.

Table 3—Summary of RMANCOVA statistical analysis for individual wall thickness measurements pooled across study sites.

Variable	Time point	Treatment group	N	LS mean	SEM	*P* values	*P* value vs placebo
Maximum LVPWd (2-D)	Pooled across days	Placebo	25	7.55	0.26	Treatment * visit: .682	—
		Low dose	**29**	**6.75***	**0.24**		**.032**
		High Dose	23	7.12	0.26		0.256
		IVP–pooled	**52**	**6.93***	**—**		**.061**
	Day 60	Placebo	13	7.48	0.29		—
		Low dose	15	6.68	0.27	**Treatment: .097**	NA†
		High dose	13	7.20	0.29		NA
	Day 180	Placebo	12	7.61	0.30		—
		Low dose	14	6.81	0.28		NA†
		High dose	10	7.03	0.32		NA
Maximum IVSd (2-D)	Pooled across days	Placebo	25	6.64	0.17	Treatment * visit: .701	NA
		Low dose	29	6.74	0.16		NA
		High dose	23	6.81	0.18		NA
	Day 60	Placebo	13	6.74	0.21	Treatment: .773	NA
		Low dose	15	6.70	0.20		NA
		High dose	13	6.78	0.21		NA
	Day 180	Placebo	12	6.53	0.22		NA
		Low dose	14	6.78	0.21		NA
		High dose	10	6.83	0.25		NA
Maximum IVSd (M-mode)	Pooled across days	Placebo	25	6.13	0.21	Treatment * visit: .933	NA
		Low dose	29	6.05	0.20		NA
		High dose	23	6.27	0.23		NA
	Day 60	Placebo	13	5.99	0.27	Treatment: .779	NA
		Low dose	15	6.00	0.26		NA
		High dose	13	6.18	0.27		NA
	Day 180	Placebo	12	6.27	0.28		NA
		Low dose	14	6.10	0.27		NA
		High dose	10	6.35	0.31		NA
Maximum LVPWd (M-mode)	Pooled across days	Placebo	25	7.16	0.28	Treatment * visit: .291	NA
		Low dose	29	6.81	0.26		NA
		High dose	23	6.98	0.28		NA
	Day 60	Placebo	13	6.82	0.34	Treatment: .663	NA
		Low dose	15	6.85	0.32		NA
		High dose	13	6.65	0.34		NA
	Day 180	Placebo	12	7.51	0.35		NA
		Low dose	14	6.76	0.33		NA
		High dose	10	7.31	0.38		NA

Statistically significant ($P < .1$) values are bolded.
†Not applicable. The treatment by visit interaction was not significant; comparisons pooled across visits were made.
See Tables 1 and 2 for the key.

Baseline, day 60, and day 180 echocardiographic data are presented as arithmetic means and SDs **(Supplementary Table S8)**. The percent of animals with a change from screening in MWT of > 0.5 mm was not significantly different between treatment groups (Supplementary Table S1).

The relationship between baseline MWT and NTproBNP values to progression/response was evaluated to determine whether baseline parameters were useful predictors of treatment outcome. A statistically significant relationship between NTproBNP values at baseline and change in MWT from baseline to day 180 was observed. Higher baseline NTproBNP values were positively correlated (r^2 = 0.26; *P* = .001) with a change in MWT from baseline **(Figure 2)**.

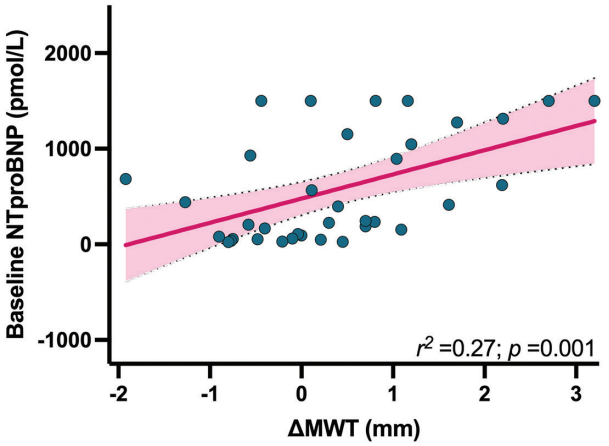

Figure 2—Pearson correlation of baseline N-terminal pro-B-type natriuretic peptide (NTproBNP) with change in MWT (mm) from baseline to day 180. The best-fit line is shown in bright pink. The 95% CI is shaded in light pink.

When placebo- and rapamycin-treated groups were compared, a more pronounced MWT progression from baseline to day 180 in cats receiving placebo with baseline NTproBNP > 100 pmol/L was noted, although this did not reach statistical significance (*P* = .11; **Figure 3**). Neither baseline MWT nor baseline cTNI predicted change in MWT at day 180 (r^2 = 0.09, *P* > .23; and r^2 = 0.11, *P* > .31, respectively).

Midway into the study period, previously used US CTnI kits were discontinued by the manufacturer, Siemens Healthineers, and no longer available for use by the reference laboratory (Texas A&M Gastrointestinal Laboratory); for this reason, subsequent analyses were conducted using an HS kit. Mean baseline results for US kits were 0.63 ng/mL (range, 0.05 to 1.84 ng/mL), 0.57 ng/mL (range, 0 to 1.9 ng/mL), and 0.60 ng/mL (range, 0 to 1.98 ng/mL) for low-dose, high-dose, and placebo groups, respectively. At day 180, mean values using HS kits were numerically higher in the placebo group at 491.45 pg/mL (range, 14.63 to 1,996.57 pg/mL) than in the low-dose group at 254.39 pg/mL (range, 9.01 to 808.6 pg/mL) and in the high-dose group at 244.03 pg/mL (range, 15.02 to 445.9 pg/mL), but these differences did not reach statistical significance (*P* = .25).

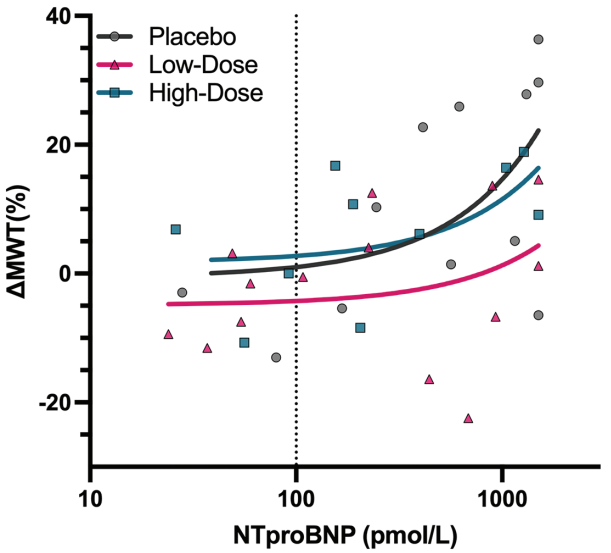

Figure 3—Baseline NTproBNP values and MWT changes (%) from baseline to day 180 in placebo (gray) and low- (pink) and high-dose (teal) delayed-release rapamycin groups. Color-matched curved best-fit lines are shown for each dose group by use of a nonlinear regression model and least-squares method in Prism (version 9.0; GraphPad Software). A dashed vertical line at 100 pmol/L denotes the cut point used for an abnormal NTproBNP in cats.

Discussion

This was the first study evaluating the effects of rapamycin in cats with subclinical HCM. Furthermore, this study evaluated these effects in the absence of observed pressure overload. The data presented here demonstrate that once-weekly low-dose (0.3 mg/kg) DR rapamycin significantly alters the primary outcome variable, MWT, over a 6-month period. Furthermore, oral low- and high-dose DR rapamycin were well tolerated with no significant differences in adverse effects compared to placebo. Therefore, results of this study suggest that this new formulation of rapamycin may effectively prevent or delay progression of LV hypertrophy in cats with subclinical HCM, establishing DR rapamycin or inhibition of the mTOR pathway as the first promising new approach for treatment of subclinical feline HCM.

Similar to our findings, previous in vitro and in vivo studies in mouse and rat models, as well as humans, have also reported either regression or reduction of LV remodeling in response to rapamycin administration, although the vast majority of these studies evaluated LV hypertrophy in the context of pressure overload.[6–9,21] Nonetheless, investigations that evaluated the effects of rapamycin on LV hypertrophy following aortic banding in mice consistently demonstrated regression of LV cardiac remodeling assessed both echocardiographically and histologically compared to mice receiving a vehicle control.[6–8,21] Mice receiving rapamycin had an attenuated increase in cardiomyocyte size and heart weight and a reduction in interstitial fibrosis. These studies also demonstrated a normalization of gene expression altered by

pressure overload–induced LV hypertrophy.[7,8] Most notably, while aortic banding led to increased expression of *MYH7* and reduced expression of *SERCA2a*, rapamycin-treated mice reverted to normal *SERCA2a* expression and enhanced expression of *MYH7* and *MYH6* compared to their vehicle-treated controls.[7] These studies showed that rapamycin therapy was associated with a reduction in several downstream targets of the mTOR pathway important in translation and protein synthesis, including p70 ribosomal S6 kinase (S6K1) activity in the acute and chronic setting[6–8] and eukaryotic translation initiation factor-4E in the chronic setting.[8] Interestingly, when S6K1 activity was evaluated in rapamycin-treated rats that received aortic constriction or sham procedure, both groups had a reduction in S6K1 activity compared to vehicle-treated controls, suggesting that rapamycin appears to target pathways involved in LV hypertrophy by mechanisms independent of the effects of pressure overload alone.[21]

Finally, in humans, the effects of rapamycin on LV hypertrophy in kidney transplant recipients have been explored.[10] Although calcineurin inhibitors (cyclosporine or tacrolimus) are most commonly used, some patients do not tolerate these medications due to calcineurin-inhibitor–induced nephrotoxicity. A 2008 study[10] demonstrated significantly reduced LV mass index and IVSd wall thickness in renal transplant recipients receiving rapamycin over the course of 1 year compared to those receiving cyclosporine or tacrolimus. Although these patients were also receiving antihypertensive therapy with similar BPs achieved, significant regression in LV mass index and IVSd measures were only observed in patients on rapamycin therapy. This suggests that rapamycin acted through mechanisms independent of hemodynamic load.

While rapamycin-specific in vivo effects on cardiac health in cats have not been previously evaluated to the authors' knowledge, a feline model of induced right ventricular pressure overload demonstrated that many of the downstream pathways of mTOR activation are involved in feline right ventricular hypertrophy, further emphasizing that rapamycin may be a beneficial target for feline LV hypertrophy, whether it be due to pressure overload or primary HCM.[22] Furthermore, feline cardiomyocytes have been evaluated in vitro, in which rapamycin effectively downregulates or augments downstream targets of mTOR.[23,24]

It is noteworthy that a significant effect of rapamycin on LV hypertrophy was seen in the low-dose rapamycin group as opposed to the high-dose treatment group. This is postulated to be due to the dosing strategy utilized. Intermittent and low-dose administration of rapamycin has been shown to partially inhibit mTORC1 without impacting mTORC2, therefore maximizing its effects on LV remodeling while minimizing adverse side effects secondary to mTORC2 inhibition.[5,12,13] Inhibition of mTORC2 has been shown to result in glucose intolerance and hepatic insulin resistance.[13] In the current study, a single cat with risk factors for DM (obese body condition and prescribed a satiety diet) at baseline went on to develop overt DM and DKA during the study period, suggesting that mTORC2 susceptibility to inhibition may be dependent on individual patient factors. This cat had changes on day 120 bloodwork and urinalysis consistent with a diagnosis of DM that were not initially recognized by the clinician. Per the study protocol, discontinuation of therapy and removal of the cat from the study should have occurred at this point, as glucose intolerance is a known potential effect of rapamycin in other species. The occurrence of DKA in this cat on the current study suggests that caution is warranted in administering rapamycin to feline patients with risk factors for DM and that patients should be carefully monitored during dosing for development of DM. Overall, more cats in the treatment groups experienced serious adverse events compared to the placebo group; however, this did not reach statistical significance. Aside from the aforementioned patient with DKA, all other serious adverse events represented reported disease progression (CHF and sudden death) in patients with advanced, subclinical HCM. Importantly, activity of mTORC1 and mTORC2, as well as their downstream targets, were not directly measured, and as such, this hypothesis cannot be entirely confirmed.

In addition to the study's primary outcome variables, evaluation of baseline characteristics that may predict response to therapy were explored. This is of particular interest in feline HCM, as previous studies have documented some cats with little progression over the lifetime of this disease.[4] In this dataset, NTproBNP was moderately correlated with response to therapy by change in MWT over time, while baseline cTNI and baseline MWT were not significant. Unfortunately, due to a change in methodology with the reference laboratory for measurement of cTNI during the study period, exploratory evaluation of cTNI was limited to baseline assessment. Future pivotal studies should aim to further explore these relationships to predict the most robust drug responders.

It is important to evaluate our findings considering certain limitations. Our primary end point of altered MWT was chosen on the basis of the known pathophysiology of HCM and prior studies demonstrating favorable efficacy in the management of maladaptive LV hypertrophy. Future pivotal studies investigating the impact of rapamycin on previously observed prognostic markers of feline HCM are warranted. Additionally, this study only evaluated dosing of DR rapamycin in subclinical cats with HCM over a 6-month period without evidence of significant LVOTO. It is unclear how rapamycin will impact this population of cats over a longer period of time or in cats with significant LVOTO, although animal models of pressure overload would suggest that cats with LVOTO would likely benefit from this drug as well.[6–8] Further studies are required to determine whether the benefits observed in this study ultimately result in a prolonged life-span, reduced risk of complications associated with HCM (ie, CHF, ATE, arrhythmia generation, and sudden death), and improved QOL. Furthermore, whether rapamycin will have a similar impact if started in patients after development of one

of these severe outcomes remains unclear. A study[7] of mice with LV hypertrophy due to aortic banding showed improved LV remodeling in mice with both compensated and decompensated LV hypertrophy, suggesting that rapamycin may be beneficial in both the subclinical and symptomatic phases of disease. The precise mechanisms of action of the drug on the hypertrophied LV myocardium remain unknown; future studies interrogating drug impact on gene and protein expression at the level of the feline heart are warranted. Finally, while we utilized statistical testing to minimize differences between groups at baseline and a *P* value threshold that reflected the exploratory nature of this initial nonpivotal study, the possibility of a type I error must be considered.

It is important to address the remarkable heterogeneity of genotypic and phenotypic expression of feline HCM and how this may impact the effect of rapamycin in a larger population of affected cats. While this may have impacted our results to some degree, there still appears to be a clear treatment effect on LV hypertrophy in cats receiving low-dose rapamycin. Future studies could aim to evaluate the impact of pharmacogenetics on the individual patient's response to rapamycin. Although this nonpivotal study reached an adequate sample size to resolve statistical significance for MWT, the possibility of both type I and type II error remains.

In conclusion, this study showed that DR rapamycin is a promising novel drug for continued study of the treatment of subclinical feline HCM that may prevent or delay progression of LV hypertrophy. Results of our study demonstrated that once-weekly dosing of DR rapamycin was well-tolerated at both doses studied. The significant treatment by visit effect on MWT at any location detected in the low-dose group on day 180 supports the effectiveness of 0.3 mg/kg once-weekly DR rapamycin in reducing LV hypertrophy in feline subclinical HCM. The greater treatment effect observed in the low-dose group compared to the high-dose group may represent a type II error given the small cohort size (n = 10) in the high-dose group at day 180, or may reflect a more favorable balance of mTORC1 suppression and activity across the weekly dosing interval while minimizing long-term adverse effects via mTORC2 inhibition. Future studies are needed to elucidate the impact of this drug on long-term survival and determine whether rapamycin delays the onset of other HCM disease outcomes.

Acknowledgments

This study was funded by TriviumVet (Waterford, Ireland). Authors LG, AF, SF, and SK are employed by TriviumVet. JAS serves as a consultant and scientific advisory board member for TriviumVet. None of the industry sponsors had any influence into the final results or conclusions. Support for JLK was provided in part by NIH T32 OD011147 and Morris Animal Foundation D22FE-027. Support for VNR was provided in part by NIH T32 HL086350 and TL1 TR001861 as well as through TriviumVet sponsored research.

The authors gratefully acknowledge the owners and cats who participated in this study, expert statistical support of Steve Radecki, expert study monitoring through Argenta Global (New Brunswick, New Jersey), as well as the contributions of the technical staff, cardiology residents, and cardiologists at the University of California-Davis School of Veterinary Medicine and North Carolina State University College of Veterinary Medicine.

References

1. Kittleson MD, Côté E. The feline cardiomyopathies: 2. hypertrophic cardiomyopathy. *J Feline Med Surg*. 2021;23(11):1028–1051. doi:10.1177/1098612X211020162
2. Luis Fuentes V, Abbott J, Chetboul V, et al. ACVIM consensus statement guidelines for the classification, diagnosis, and management of cardiomyopathies in cats. *J Vet Intern Med*. 2020;34(3):1062–1077. doi:10.1111/jvim.15745
3. Payne JR, Brodbelt DC, Luis Fuentes V. Cardiomyopathy prevalence in 780 apparently healthy cats in rehoming centres (the CatScan study). *J Vet Cardiol*. 2015;17(suppl 1):S244–S257. doi:10.1016/j.jvc.2015.03.008
4. Fox PR, Keene BW, Lamb K, et al. International collaborative study to assess cardiovascular risk and evaluate long-term health in cats with preclinical hypertrophic cardiomyopathy and apparently healthy cats: the REVEAL Study. *J Vet Intern Med*. 2018;32(3):930–943. doi:10.1111/jvim.15122
5. Sciarretta S, Forte M, Frati G, Sadoshima J. New insights into the role of mTOR signaling in the cardiovascular system. *Circ Res*. 2018;122(3):489–505. doi:10.1161/CIRCRESAHA.117.311147
6. Shioi T, McMullen JR, Tarnavski O, et al. Rapamycin attenuates load-induced cardiac hypertrophy in mice. *Circulation*. 2003;107(12):1664–1670. doi:10.1161/01.CIR.0000057979.36322.88
7. McMullen JR, Sherwood MC, Tarnavski O, et al. Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation*. 2004;109(24):3050–3055. doi:10.1161/01.CIR.0000130641.08705.45
8. Gao XM, Wong G, Wang B, et al. Inhibition of mTOR reduces chronic pressure-overload cardiac hypertrophy and fibrosis. *J Hypertens*. 2006;24(8):1663–1670. doi:10.1097/01.hjh.0000239304.01496.83
9. Marin TM, Keith K, Davies B, et al. Rapamycin reverses hypertrophic cardiomyopathy in a mouse model of LEOPARD syndrome-associated PTPN11 mutation. *J Clin Invest*. 2011;121(3):1026–1043. doi:10.1172/JCI44972
10. Paoletti E, Amidone M, Cassottana P, Gherzi M, Marsano L, Cannella G. Effect of sirolimus on left ventricular hypertrophy in kidney transplant recipients: a 1-year nonrandomized controlled trial. *Am J Kidney Dis*. 2008;52(2):324–330. doi:10.1053/j.ajkd.2008.04.018
11. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012;149(2):274–293. doi:10.1016/j.cell.2012.03.017
12. Arriola Apelo SI, Lamming DW. Rapamycin: an inhibiTOR of aging emerges from the soil of Easter Island. *J Gerontol A Biol Sci Med Sci*. 2016;71(7):841–849. doi:10.1093/gerona/glw090
13. Arriola Apelo SI, Pumper CP, Baar EL, Cummings NE, Lamming DW. Intermittent administration of rapamycin extends the life span of female C57BL/6J mice. *J Gerontol A Biol Sci Med Sci*. 2016;71(7):876–881. doi:10.1093/gerona/glw064
14. Arriola Apelo SI, Neuman JC, Baar EL, et al. Alternative rapamycin treatment regimens mitigate the impact of rapamycin on glucose homeostasis and the immune system. *Aging Cell*. 2016;15(1):28–38. doi:10.1111/acel.12405
15. Urfer SR, Kaeberlein TL, Mailheau S, et al. A randomized controlled trial to establish effects of short-term rapamycin treatment in 24 middle-aged companion dogs. *Geroscience*. 2017;39(2):117–127. doi:10.1007/s11357-017-9972-z
16. Freeman LM, Rush JE, Oyama MA, et al. Development and

evaluation of a questionnaire for assessment of health-related quality of life in cats with cardiac disease. *J Am Vet Med Assoc*. 2012;240(10):1188–1193. doi:10.2460/javma.240.10.1188
17. Langford V. *TriviumVet. Tolerance of Rapamycin in Cats.* Kingfisher International Inc; 2020.
18. LeBlanc AK, Atherton M, Bentley RT, et al. Veterinary Cooperative Oncology Group–Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) following investigational therapy in dogs and cats. *Vet Comp Oncol*. 2021;19(2):311–352. doi:10.1111/vco.12677
19. Abbott JA, MacLean HN. Two-dimensional echocardiographic assessment of the feline left atrium. *J Vet Intern Med*. 2006;20(1):111–119. doi:10.1892/0891-6640(2006)20[111:teaotf]2.0.co;2
20. Maerz I, Schober K, Oechtering G. Echocardiographic measurement of left atrial dimension in healthy cats and cats with left ventricular hypertrophy. *Tierarztl Prax Ausg K Kleintiere Heimtiere*. 2006;34:331.
21. Boluyt MO, Li ZB, Loyd AM, Scalia AF, Cirrincione GM, Jackson RR. The mTOR/p70S6K signal transduction pathway plays a role in cardiac hypertrophy and influences expression of myosin heavy chain genes in vivo. *Cardiovasc Drugs Ther*. 2004;18(4):257–267. doi:10.1023/B:CARD.0000041245.61136.56
22. Laser M, Kasi VS, Hamawaki M, Cooper G IV, Kerr CM, Kuppuswamy D. Differential activation of p70 and p85 S6 kinase isoforms during cardiac hypertrophy in the adult mammal. *J Biol Chem*. 1998;273(38):24610–24619. doi:10.1074/jbc.273.38.24610
23. Tuxworth WJ Jr, Wada H, Ishibashi Y, McDermott PJ. Role of load in regulating eIF-4F complex formation in adult feline cardiocytes. *Am J Physiol*. 1999;277(4):H1273–H1282. doi:10.1152/ajpheart.1999.277.4.H1273
24. Moschella PC, McKillop J, Pleasant DL, Harston RK, Balasubramanian S, Kuppuswamy D. mTOR complex 2 mediates Akt phosphorylation that requires PKCε in adult cardiac muscle cells. *Cell Signal*. 2013;25(9):1904–1912. doi:10.1016/j.cellsig.2013.05.001

Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org