CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214998Orig1s000

NON-CLINICAL REVIEW(S)

Memorandum

Division of Pharmacology/Toxicology
Office of Cardiology, Hematology, Endocrinology, & Nephrology
Center for Drug Evaluation and Research

NDA SECONDARY REVIEW

Date:	5 October 2021
NDA #	214998
Applicant:	MYOKARDIA INC
Drug:	Mavacamten (cardiac myosin inhibitor)
Primary Reviewer:	Gowra Jagadeesh, Ph.D
Secondary Reviewer:	Xuan Chi, Ph.D.

MyoKardia INC is seeking market approval for mavacamten, proposed trade name CAMZYOS, as a treatment option for symptomatic obstructive hypertrophic cardiomyopathy (HCM) in adults. HCM is a genetically determined cardiac muscle disease most often caused by mutations in one of several sarcomere genes. The hallmark of HCM is left ventricular hypertrophy and hypercontractility accompanied by reduced left ventricular compliance. Mavacamten is a small molecule inhibitor of cardiac myosin. It reversibly inhibits the binding of cardiac myosin to actin, stabilizing this off-actin state and reducing the number of myosin motors engaging the actin filament. This results in reduced aggregate contractile force during cardiac systole and reduced residual cross-bridges during diastole, providing a mechanistic basis to reduce contractility, and improve diastolic relaxation as well as outflow tract obstruction in patients with HCM. Mavacamten would be a first-in-class cardiac myosin inhibitor, should it be approved. We agree with using "cardiac myosin inhibitor" as the Established Pharmacologic Class (EPC) for mavacamten, as this term is supported by the mechanism of action of the drug, and it is clinically meaningful and scientifically valid.

Dr. Jagadeesh, the primary nonclinical reviewer, concludes that the pharmacology and toxicology data support approval of mavacamten. *I concur with Dr. Jagadeesh's assessment.*

The mechanism of action of mavacamten has been demonstrated by in vitro, ex vivo and in vivo pharmacology studies in multiple species, including healthy animals, animal models of HCM and purified recombinant proteins of human HCM mutations. Mavacamten is slightly more potent in the species used for general toxicological assessment, and comparable potencies were exhibited by the recombinant wild type human beta cardiac myosin and five HCM mutants. The pharmacologically active dose (PAD) obtained from a single oral dose study in telemetered healthy rat was 1 mg/kg/day, which reduced fractional shortening (FS) by approximately 20%. In the dog, 0.045 mg/kg mavacamten for 31 days produced approximately 11% reduction in FS.

In the toxicology studies, mavacamten elicited expected pharmacological effects from inhibiting cardiac myosin, demonstrated by dose-dependent reduction in cardiac contractility culminating in cardiac failure or unintended deaths. Mortalities occurred at progressively lower doses with longer study durations. Additional findings in other organs were mostly secondary effects of

heart failure. A no-observed-adverse-effect-level (NOAEL) was determined to be 0.3 mg/kg/day and 0.06 mg/kg/day, respectively for the rat and dog, based on echocardiographic, electrocardiographic, or histological endpoints in the heart in the chronic toxicity studies. It is uncertain whether pharmacological activity was present at these NOAEL doses, though a reduction in FS or ejection fraction of less than 10% may have been present in both species. At the highest doses, which are in fact similar to or lower than clinical exposures, unequivocal reductions were observed in cardiac contractility consistent with the intended pharmacologic effect of mavacamten, which was tolerated in dogs but lead to heart failure-related deaths in rats over a chronic period. Ejection fractions were reduced ~30% in both rats and dogs at this highest dose level. There is a narrow therapeutic window between the NOAEL and higher doses that substantially impacted cardiac contractility; in general, pharmacologically active doses largely overlap with doses that produce some toxicities. The dog seems to be the more sensitive species to the toxic effects of mavacamten, as the drug has higher bioavailability (87.1%) and longer half-life (161 h) in this species.

Accumulation of the study drug was noted with repeated daily oral dosing, with accumulation ratios up to 2.8-fold in rats and 9-fold in dogs. The elimination half-life (t1/2) was long for dogs (161 h) and relatively short for rats (8 h), after a single oral administration. The slow rise in drug concentration due to accumulation appeared to allow dogs to tolerate the cardiac effects of mavacamten at a level that was less tolerated if experienced upon acute exposure. This suggests an active process of compensation might occur as concentrations of mavacamten increase toward a steady state.

Mavacamten was found to be teratogenic in both rats and rabbits at clinically relevant exposures based on the maximum recommended human dose (MRHD, 15 mg/day). In all toxicity studies (except carcinogenicity studies), plasma exposure (Cmax and AUC) at the NOAEL is lower than that in humans at the MRHD (Table 1), suggesting potential risk at the therapeutic dose range.

The following summarizes key issues that arose during review of the nonclinical program of mavacamten.

Cardiac effects

A consistent finding in all repeat-dose toxicity studies (up to 26 weeks in rats and up to 39 weeks in dogs) was dose-dependent reduction in cardiac contractility. Cardiac toxicity resulting in heart failure and death was noted at maximum plasma concentrations of 725 ng/ml (at a dose of 1.2 mg/kg/day in 26-week toxicity study, with an exposure margin of 0.8-fold relative to that in human at MRHD) in rats and 911 ng/ml in dogs (at a dose of 0.45 mg/kg/day in 13-week toxicity study, with an exposure margin of 0.8 to 1.1-fold relative to that in human at MRHD). The cardiac toxicities were exemplified by markedly decreased blood pressure, elevated plasma levels of NT-proBNP, greater absolute and relative heart weights, and echocardiogram findings of increased heart size and reduced systolic functions. Histopathology findings of note included myocardial degeneration, inflammation and/or hypertrophy, endocardial degeneration and necrosis, osseous metaplasia of cardiac muscles, atrial thrombus, and dilation. The toxicities observed in other organs (e.g., pulmonary edema, liver congestion with centrilobular necrosis, pancreatic edema, pre-renal azotemia) were secondary effects of cardiac failure. All these findings were reversible except for partial reversibility in the heart histopathology.

In vitro and in silico electrophysiological data indicate low torsadogenic/proarrhythmogenic risk with mavacamten, as shown by the lack of an effect on hERG channel or any action potential parameters. However, sustained exposure of rats and dogs to mavacamten (at levels producing

moderate to marked functional cardiac depression for at least 7 days) were accompanied by modest ($P < 0.05$) and reversible QTc prolongation, at lower exposure (0.3 to 0.4-fold) than in human at the MRHD (Table 1). This was speculated to be an electrophysiological adaptive response to sustained myosin inhibition in ventricles, which seems plausible.

Both rat and dog studies show that mavacamten has a narrow separation (4-fold in rats and 2.5-fold in dogs) between the NOAEL and the dose that caused heart failure and mortality, indicative of a narrow therapeutic window. A plot between measured plasma concentrations and FS in rats show a non-linear but dose-dependent FS with no plateau in effect, posing a risk of fast deterioration of cardiac contractility as exposures increase. Interpretation of the exposure/response demonstrated in the nonclinical studies needs to consider the following key considerations when assessing human risk: firstly, mavacamten is slightly more potent in rats and dogs compared to humans (~2-fold based on IC50), suggesting a potential rightward shift in exposure/response in human subjects, and secondly, the indicated patient population has a hypercontractile myocardium and would likely tolerate a reduction in cardiac contractility to a greater extent than in animals or humans with 'normal' cardiac contractility. Thus, while the nonclinical studies clearly demonstrate that mavacamten is capable of disrupting myosin/actin interactions in a dose- and time-dependent manner that, in excess, can precipitate heart failure, a broader therapeutic window may be present in the intended patient population.

Reproductive effect

Mavacamten was shown to be teratogenic in both rats and rabbits in embryo-fetal developmental studies. In rats, mavacamten increased post-implantation loss, lowered mean fetal body weight, slightly reduced fetal skeletal ossification, induced heart malformations (total situs inversus), and increased the incidence of skeletal malformations relative to control. In rabbits, increased incidences of cleft palate, great vessel malformations (dilatation of pulmonary trunk and/or aortic arch), and fused sternebrae in fetuses were observed at the same doses that caused maternal toxicity. Based on these data, mavacamten has a high likelihood of being a teratogen when administered during gestation in humans. It is not known whether mavacamten is secreted into the milk. Mavacamten did not affect fertility of male or female rats or the fertility of F1 offspring of female rats dosed with mavacamten during gestation. Plasma exposures in all reproductive toxicology studies at the NOAEL is lower than those in humans at the MRHD (Table 1).

Table 1. A summary of exposure margins based on pivotal toxicology studies with mavacamten. Animal to human exposure ratios is calculated based on MRHD of 15 mg/day
(Adopted from the primary Pharmacology and Toxicology Review by Dr. Jagadeesh)

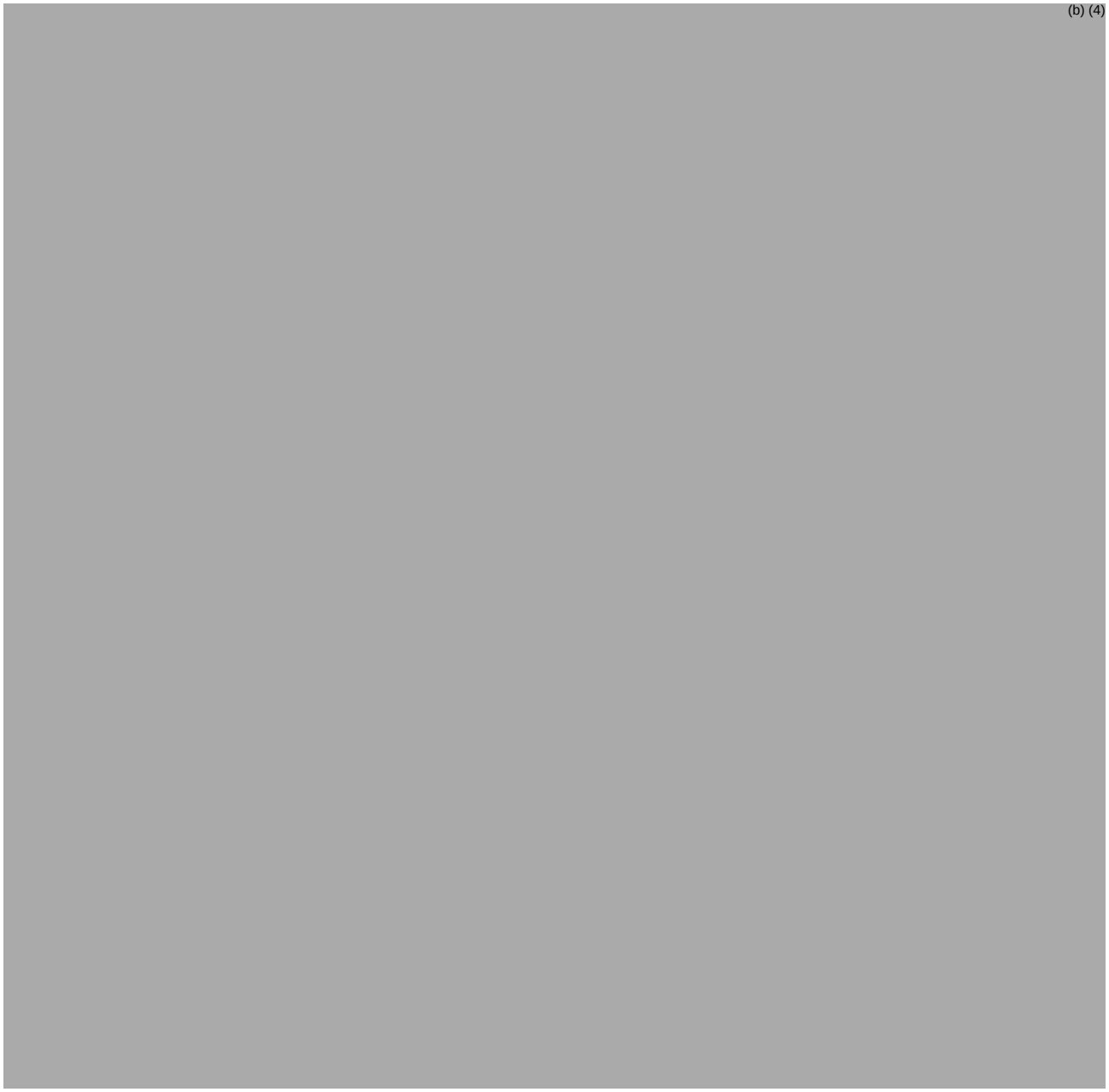
Species, GLP Tox study	Dose, (mg/kg/day)	Effects		PK data-Tox study		Multiple, Animal/human[1]	
		Significant findings	Parameter	Cmax (ng/ml)	AUC (ng.hr/ml)	By Cmax	By AUC
Rat, 26-wk[2]	**0.3**		**NOAEL**	120	1670	0.12X	0.1X
	1.2	Deaths from HF, extensive increase in heart size and a reduction in systolic function	Death	725	10700	0.80X	0.63 X
Dog 13-/39-week[3]	**0.06**		**NOAEL**	120	1580	0.12X	0.09X
	0.18	QTc interval prolongation[4]		M 388 F 288	M 6220 F 3800	0.40X 0.30X	0.37X 0.22
	0.45	Cardiac toxicity (severe ventricular dilation) resulting in heart failure. QTc interval prolongation[4]	Death	M1040[5] F 782[6]	M 17200 F 12300	1.10X 0.81X	1.02X 0.73X
Carcinogenicity							
RasH2 mouse[7]	M 2 F 3	No tumorigenic findings	**NOAEL**	M 2230 F 4280	M 30000 F 50400	2.30X 4.45X	1.78X 3.00X
Fertility							
Rat	1.2	Fertility and early embryonic development to implantation	**NOAEL**	NA	NA	-	-
Embryo-fetal Development Toxicity							
Rat[8]	1.5	Teratogenic potential[9]		1080	16500	1.12X	0.98X
	0.75	Developmental	**NOAEL**	356	5690	0.40X	0.34X
	1.5	Maternal	**NOAEL**	1080	16500	1.12X	0.98X
Rabbit[8]	1.2	Teratogenic potential		1100	16500	1.14X	0.98X
	0.6	Maternal and developmental	**NOAEL**	516	7160	0.54X	0.42X
Pre- and Postnatal Development Toxicity							
Rat[10]	1.5	F_0 Maternal, F_1 developmental and reproductive, F_2 embryonic	**NOAEL**	1080	16500	1.12X	0.98X

Male and female combined mean Cmax and AUC0-24 values were used unless specified. M: male; F: female
1: Based on EXPLORER-HCM study at a dose of 15 mg MYK-461 (mavacamten)/day for 10 days. Mean Cmax: 962 ng/ml, AUC_{0-24} 16,891 h*ng/mL used for calculating exposure multiples (Sponsor communication).
2: PK data measured on day 182
3: PK data from 13-week study, measured on day 73 for males at 0.45 mg/kg/day and the rest on day 86.
4: 39-week study: ECG evaluation showed prolongation ($P < 0.05$) of QTc intervals in animals receiving ≥ 0.18 mg/kg/day relative to predose and control values. Echocardiogram evaluations indicated substantial increases in mean LV end-diastolic and a substantial reduction in mean LV ejection fraction.
5: Two males died/euthanized on days 70, 72
6: One female euthanized on day 93
7: TK measurements on day 182
8: Exposure data in reproductive toxicity were based on gestation day 12
9: At 1.5 mg/kg/day, increased post-implantation loss, altered fetal growth (such as lower fetal body weight and reduced fetal ossification of bones (cervical and thoracic vertebrae and), visceral (heart) malformations and skeletal malformations were observed.
10: Plasma concentrations of MYK-461 were not assessed in this study. Exposure multiples were based on the PK data in Embryo-fetal development study in rats.

Pregnancy and Lactation
Mavacamten was shown to cause visceral and skeletal malformations in both rats and rabbits and to increase post-implantation loss in rats, when administered during organogenesis at exposures close to the MRHD. In rats, the embryofetal toxicities were observed in the absence of maternal toxicities, while in the rabbits, they were concurrent with maternal toxicities.

Due to the severity of these findings and their presence in two species at exposures close to that at MRHD, the primary reviewer and I both consider these findings informative of human risk and should be disclosed in the drug label, under Section 8.1 as part of the Risk Summary and Animal Data, as well as under Section 5.4 "Fetal Toxicity" as part of the Warmings and Precautions.

The suggested language relevant to embryofetal toxicities in Section 5.4 and 8.1 of the drug label are included below:



(b) (4)

Genotoxicity and Carcinogenicity

A standard battery of genotoxicity studies (Ames assay, in vitro chromosome aberration assay in human lymphocytes, and in vivo rat micronucleus assay in bone marrow) were all negative. A 26-week oral gavage carcinogenicity study was conducted in the RasH2 transgenic mouse to determine the carcinogenic potential of mavacamten and the results show that there were no drug-related neoplasms in either males or females in this study. This conclusion was concurred by CDER Executive Carcinogenicity Assessment Committee and is included in Section 13.1 of the drug label.

The Division has agreed the 2-year rat carcinogenicity study can be completed post-approval and the study is currently ongoing.

The suggested language under Section 13.1 of the drug label related to genotoxicity and carcinogenicity is included below:

(b) (4)

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

XUAN CHI
10/05/2021 09:46:46 AM

TODD M BOURCIER
10/05/2021 09:56:53 AM
I concur with Dr. Chi's conclusions and recommendations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	214998
Supporting document/s:	0001, 0034
Applicant's letter date:	January 28, 2021
CDER stamp date:	January 28, 2021
Product:	Mavacamten (CAMZYOS®)
Indication:	Treatment of symptomatic obstructive hypertrophic cardiomyopathy
Applicant:	MyoKardia Inc
Review Division:	Pharmacology and Toxicology, OCHEN, OND
Reviewer:	G. Jagadeesh, Ph.D.
Supervisor/Team Leader:	Xuan Chi, Ph.D.
Division Director:	Todd Bourcier, Ph.D.
Project Manager:	Alexis Childers
Date of review	
Submission:	September 22, 2021

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 214998 are owned by MyoKardia Inc. or are data for which MyoKardia Inc., has obtained a written right of reference.
Any information or data necessary for approval of NDA 214998 that MyoKardia does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 214998.

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1 Executive Summary

1.1 Introduction (and Clinical Rationale)

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant genetic disease, which is defined clinically as unexplained left ventricle hypertrophy in the absence of known causes such as pressure overload, systemic diseases or infiltrative processes (Gersh et al., 2011). The hallmark of HCM is myocardial hypercontractility accompanied by reduced LV compliance. Clinically, it is diagnosed as reduced ventricular chamber size, supranormal ejection fraction and diastolic dysfunction (diminished relaxation capacity). Histopathologically, it includes myocyte hypertrophy and disarray, microvascular remodeling and fibrosis (Frey et al., 2012). In the general population, it is present in approximately 1 in 500 individuals. Among them, approximately 50% of affected individuals demonstrate point mutations in one of the structural genes of the sarcomere (the contractile unit of cardiomyocyte). Further, 25-30% of pathogenic HCM variants are found in the cardiac myosin gene, *MYH7* (Maron 2012). Symptomatic patients most commonly report exertional dyspnea, often in the absence of important LV outflow tract (LVOT) obstruction and even without severe hypertrophy. This often limits activities of daily living and can be debilitating. The patients are at increased risk for adverse clinical events including overt heart failure prompting hospitalization, atrial fibrillation, syncope, malignant ventricular arrhythmias, and sudden cardiac death (Gersh et al., 2011). Currently, the treatment consists of off-label use of beta-adrenergic blockers, non-dihydropyridine calcium channel blockers and disopyramide that indirectly blunt sarcomeric hyperactivity, alleviate LVOT obstruction, and improve symptoms. No available agent has been convincingly demonstrated to directly improve diastolic function in HCM (Sherrid 2016) and none target the underlying pathophysiology of the disease.

According to the applicant, at present there are no sarcomere-targeted therapies for HCM developed or tested. In vitro biochemical analyses suggest that disease-causing mutations in cardiac myosin increase force production relative to wild type by about 50% (Sommese et al., 2013). Thus, drugs targeting the disease at its source might influence the downstream events to reverse left ventricle remodeling back towards normal. In this direction, the sponsor contemplates that the investigative drug, mavacamten (MYK-461), a first-in-class small molecule allosteric modulator of beta cardiac myosin, selectively targets cardiac myosin and reversibly inhibits its binding to actin. This reduces the aggregate force (and thus power output) of systolic contraction and is predicted to facilitate diastolic relaxation (lusitropy or distensibility) and predicted to improve dynamic LVOT obstruction in patients with HCM.

1.2 Brief Discussion of Nonclinical Findings

Based on its intended mechanism of distensibility, mavacamten (MVA) has a high potential to induce cardiac toxicity culminating in heart failure as a result of large increase in heart rate, marked decrease in blood pressure followed by severe reduction in cardiac contractility (decrease in fractional shortening [FS] of muscle, diastolic relaxation). Both rat and dog studies show that the drug has a very narrow therapeutic index. A mere 3-fold safety margin exists between the NOAEL and mortality. Dogs could not tolerate a single i.v. dose over 1.2 mg/kg and a single oral dose over 7 mg/kg (approximately 15 times the therapeutic concentration in humans at the MRHD). In repeat dose toxicity studies, early mortality was noted in rats at 3 mg/kg/day and in dogs at 1 mg/kg/day. A plot between measured plasma concentrations and FS shows a non-linear dose-dependent FS with no plateau in effect, posing the risk of achieving exposures above 1000 ng/ml that are not well tolerated. Sustained exposure of rats and dogs to MVA (at levels producing moderate to marked functional cardiac depression for at least 7 days) were accompanied by modest ($P < 0.05$) QTc interval prolongation, which is 0.08 to 0.1 times human exposure (AUC) at the MRHD (Table 146). Thus, MVA has a low torsadogenic or proarrhythmic risk. The QTc changes were reversible upon discontinuation of treatment. Beta-adrenergic agonists (isoproterenol and dobutamine) oppose the effect by restoring the contractility to 60% of baseline. However, the reversal is transient given the long-lasting effect of mavacamten on the heart. Thus, other measures such as dialysis should be in place to overcome the sudden toxicity or excessive dosage of mavacamten.

Other target organs of toxicity in both rats and dogs were liver (centrilobular necrosis, congestion and vacuolation), lungs (increased alveolar macrophages and inflammation) and thymus (decrease in size as a result of decreased cellularity). At supratherapeutic exposures (beyond NOAEL), increases in BUN, creatinine, potassium and phosphorous concentrations that were considered reflective of pre-renal azotemia were noted in the rat and dog. Furthermore, edema and congestion were present in multiple organs and body cavities. All of these findings were reversible except for partial reversibility in the heart histopathology. Dogs are more sensitive than rats as the drug is highly bioavailable with large volume of distribution especially left ventricle to plasma ratio > 20 and long-half-life (~7 days). Repeated dosing resulted in accumulation of test substance (up to 2.8-fold in rats 9-fold in dogs).

Although MVA did not cause transgenerational reproductive effects or adversely affect fertility of male or female rats; in embryo-fetal development study in rats, MVA increased post-implantation loss, lowered mean fetal body weight, slightly reduced fetal skeletal ossification, induced heart malformation (total situs inversus), and increased

incidences of skeletal malformations relative to control. In rabbits, increased incidences of cleft palate, great vessel malformations (dilatation of pulmonary trunk and/or aortic arch), and fused sternebrae in fetuses were observed at the same doses that caused maternal toxicity. Mavacamten has a high probability of being a **teratogen when administered during gestation**. Exposure of the fetus, placenta, and amniotic fluid to MVA was demonstrated in pregnant rabbits (on Gestation Day 12). MVA was distributed in theses tissues, with individual embryonic tissue to plasma concentration ratios ranging from 0.09 to 0.15 across the dose levels. It is not known whether MVA excretes into the milk. Plasma exposure in all toxicology studies and reproductive toxicology studies at the NOAEL is less than those in humans at the MRHD (Table 146). Mavacamten was not mutagenic or genotoxic in in vivo and in vitro assays. No evidence of carcinogenic potential has been identified in the 6-month transgenic mouse study, and results from the 24-month rat study are pending.

1.3 Recommendations

1.3.1 Approvability

There are no approvability issues for mavacamten based on nonclinical toxicity testing program.

1.3.2 Additional Non-Clinical Recommendations

None currently

1.3.3 Labeling

Recommendations and edits were made on the labeling document on SharePoint and presented at the Division labeling meetings.

2 Drug Information

2.1 Drug

CAS Registry Number: 1642288-47-8

Generic Name: Mavacamten

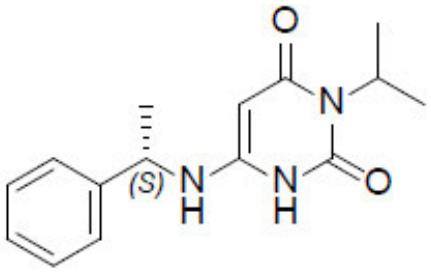
Trade Name: CAMZYOS®

Code Name: MYK-461

Chemical Name: 6-{[(1S)-1-phenylethyl]amino}-3-(propan-2-yl)pyrimidine-2,4(1H,3H)-dione

Molecular Formula/Molecular Weight: $C_{15}H_{19}N_3O_2$, 273.3

Structure or Biochemical Description: MYK-461 is a highly permeable, white to off-white crystalline solid, not hygroscopic, which is practically insoluble in water. The drug substance is manufactured by (b) (4)



mavacamten (MYK-461)

Pharmacologic Class: Cardiac myosin inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 121904, (b) (4)

2.3 Drug Formulation

Mavacamten drug product is an oral immediate release capsule, supplied in four dosage strengths, 2.5 mg, 5 mg, 10 mg, and 15 mg (maximum) in size 2 hard gelatin capsules, differentiated by the color of the capsule caps and the imprint of dosage strengths on the capsule.

2.4 Comments on Novel Excipients

No novel excipients are used in the manufacture of the mavacamten capsule drug product.

2.5 Comments on Impurities/Degradants of Concern

Risk assessments have been conducted for possible contribution of (b) (4) impurities from the excipients, drug substance, primary packaging material, and

manufacturing process of mavacamten capsules. No change in the toxicological profile of mavacamten was noted for the presence of two additional impurities (MYK-460 and [redacted] (see section 10.1 for details). MYK-460, the enantiomer of mavacamten in the drug substance is non genotoxic (see sections 7.2, 7.4). It has been shown to not formed during manufacture and stability study of the capsule drug product, and its level is controlled ([redacted]%) in the drug substance. Two degradation products [redacted] of mavacamten capsule formulations were observed at up to [redacted]% during long-term and accelerated stability studies. Both are classified as non-mutagenic Class 5 impurities per ICH M7 guidance. Total levels of elemental impurities from excipients, API and drug product are below ICH specified control limits.

Table 1. Ingredients, quality standards, pharmaceutical functions, unit compositions for mavacamten capsules

Ingredient	Quality Standard	Pharmaceutical Function	2.5 mg Capsules		5 mg Capsules		10 mg Capsules		15 mg Capsules	
			Qty per Unit (mg)	%	Qty per Unit (mg)	%	Qty per Unit (mg)	%	Qty per Unit (mg)	%
Active Ingredient										
Mavacamten	Section 3.2.S.4.1	Active	2.5	[redacted]	5.0	[redacted]	10.0	[redacted]	15.0	[redacted]
Inactive Ingredients										
Silicon dioxide	USP-NF, EP, JP	[redacted]								
Mannitol	USP-NF, EP, JP									
Hypromellose	USP-NF, EP, JP									
Croscarmellose sodium	USP-NF, EP, JP									
Magnesium stearate (non-bovine)	USP-NF, EP, JP									
Total Blend Weight per Capsule										
Size 2 hard gelatin capsule shells for 2.5 mg, 5 mg, 10 mg or 15 mg [a]	Section 3.2.P.4.1	Capsule shell	[redacted]							
Total Capsule weight			161		261		221		301	

2.6 Proposed Clinical Population and Dosing Regimen

MVA is indicated for the treatment of symptomatic obstructive hypertrophic cardiomyopathy in adults to reduce left ventricular outflow tract obstruction and ventricular filling pressures, improve symptoms, and increase exercise capacity. The recommended starting dose is 5 mg orally once daily and the maximum recommended dose is 15 mg once daily.

2.7 Regulatory Background

An IND for MYK-461 (subsequently MVA) (#121904) was submitted on October 17, 2014.

3 Studies Not Reviewed

1. Long term effects of three test articles on human stem cell-derived cardiomyocytes (field potential and impedance analysis). Report #NC-20-0038.
2. ScreenPatch® assay (IonWorks™ Barracuda Based Assay): Effects of three test articles on hERG channels expressed in mammalian cells. Report #NC-20-0039.
3. hERG-Lite assay: Effects of Test articles on cloned hERG channel surface expression in mammalian cells. Report #NC-20-0040.
4. Effects of test articles on cloned hKv4.3 channel surface expression in mammalian cells. Report #NC-20-0042.
5. Evaluation of the uptake of MYK-461 by Human Hepatocytes – Substrate Potential for Transporters. Report #NC-19-0014
6. Evaluation of MYK-461 as Inhibitor of Human Efflux Transporters P-gp, BCRP, BSEP, and Human Uptake Transporters OATP1B1, OATP1B3, OCT1 and OCT2. Report #NC-19-0017.
7. Evaluation of MYK-461 as an inhibitor of human MATE1, MATE2-K Efflux Transporters and OAT1 and OAT3 uptake transporters. Report #NC-19-0015.
8. Time-Dependent Inhibition of CYP450 Enzymes by MYK-461 in Human microsomes. Report #NC-17-0021.
9. Evaluation of Mechanism -based inhibition of CYP2C19 and CYP2D6. Report #NC-19-0018.
10. Metabolic Stability of [14C]MYK-461 in Liver Microsomes. Report #NC-17-0019.
11. Metabolic Stability of MYK-461 in Hepatocytes. Report #NC-19-0021.
12. Caco-2 Cell Permeability Assay, NC-17-0022, NC-19-0016
13. Repeat Dose Exposures of MYK-461 in Male Mice. NC-14-0016
14. In vivo enantiomeric Ratio of MYK-461 in the rat and dog, 14-2402, 14-3222

4 Pharmacology

Established Pharmacologic Class (EPC)

MVA is the first-in-class small molecule inhibitor of cardiac myosin. It reversibly inhibits the binding of cardiac myosin to actin, stabilizing this off-actin state and reducing the number of myosin motors engaging the actin filament. This results in reduced aggregate contractile power during systole and reduced residual cross-bridges during diastole. The proposed EPC for MVA is cardiac myosin inhibitor, which is a new EPC. This term is based on the mechanism of action of MVA, which is supported by the following in vitro, ex-vivo and in vivo pharmacology studies submitted in this application.

4.1 Primary Pharmacology

4.1.1. *In vitro* studies

4.1.1.1 Inhibition of myosin in various species and selectivity for cardiac versus skeletal forms of myosin by MYK-461

This non-GLP study (#NC-14-0005) was conducted at MyoKardia, Brisbane, CA (dated 12/19/20020). MYK-461 was characterized in sarcomeric systems of increasing complexity, ranging from acto-myosin systems to myofibrils derived from cardiac, skeletal, and smooth muscles of various species. Also, to understand the effect of MYK-461 on the individual kinetic steps of the myosin cycle, transient kinetic experiments were performed to assess the effects on ATP binding, ADP release, weak-to-strong transition, and phosphate release rates of myosin.

Methods

The following studies were conducted.

- Cardiac specificity (for bovine and human cardiac muscle) of MYK-461's action was established by evaluating its effect on other myosin isoforms fast-twitch (rabbit psoas) and slow-twitch (bovine masseter) skeletal muscle as well as smooth-muscle (chicken gizzard)
- Multiple mutant myosins (R403Q, R453C, R719W, R723G, and G741R) were assayed
- Steady-state ATPase measurements in the presence of varying calcium concentrations were determined for bovine, human, dog, rat, and mouse cardiac myofibrils.
- The reversibility of mavacamten binding was performed using the actomyosin version of the steady-state assay.

- Transient kinetic experiments were performed on bovine cardiac S1 to assess the effects on ATP binding, ADP release, weak-to-strong transition, and phosphate release rates of myosin.

Results

The effect of MYK-461 on myosin in systems of varying complexity was evaluated by measuring the steady-state myosin-mediated conversion of ATP to ADP (ATPase rate) using a coupled-enzyme approach and standard Michaelis-Menten kinetic methods. MYK-461 potently inhibited bovine cardiac myosin activity in basal (motor alone) conditions (IC50, 0.27 µM), establishing the myosin-S1 motor domain as the minimal functional unit of the sarcomere. Inhibition of ATP turnover was also noted in all more complex systems tested, the acto-myosin system containing myosin-S1 and actin, MYK-461 slowed ATPase activity to a similar degree (IC50, 0.47 µM). Similar observations were made in two systems incorporating the Ca^{2}+-dependent activation characteristic of an intact sarcomere: cardiac myosin reconstituted with Ca^{2}+-regulatory soluble thin-filament proteins (IC50, 0.53 µM) and skinned ventricular myofibrils (IC50, 0.49 µM) (Table 2). These inhibitory effects were reversible, as rapid dilution restored ATPase activity in a bovine acto-myosin system.

Table 2. Half-maximal inhibitory concentrations (IC50 mean, µM ± SD) of MYK-461 on the enzymatic activity (ATPase) of myosin in systems of varying complexity

System[a]	Ca^{2+}-free		Ca^{2+}-dependent (at pCa50)	
	Basal	Actomyosin	Regulated	Myofibril
Bovine cardiac	0.27 ± 0.02	0.47 ± 0.01	0.53 ± 0.02	0.49 ± 0.03
Human cardiac	0.52 ± 0.09	0.73 ± 0.05	0.53 ± 0.08	0.71 ± 0.06
Bovine masseter (slow skeletal)	ND	ND	ND	0.43 ± 0.04
Rabbit psoas (fast skeletal)	4.71 ± 0.20	5.85 ± 0.15	5.09 ± 1.22	2.14 ± 0.31
Chicken gizzard (smooth)	> 50	> 50	ND	ND

Ca^{2+}: calcium ion; S1: myosin subfragment-1.

[a] Basal (myosin-S1 alone): bovine cardiac (0.05 µM bcS1), human (0.1 µM hcS1), rabbit fast-skeletal (0.05 µM rsS1), and chicken gizzard (0.05 µM cgS1); acto-myosin (myosin-S1 + 14 µM actin): bovine cardiac (0.25 µM bcS1), human (0.25 µM hcS1), rabbit fast-skeletal (0.1 µM rsS1), and chicken gizzard (0.5 µM cgS1); regulated (myosin-S1 + 14 µM actin + specific regulatory proteins) at pCa50 (50% of maximal activation): bovine cardiac (pCa 6.0, 0.5 µM bcS1), human (pCa 6.0, 0.5 µM hcS1), and rabbit fast-skeletal (pCa 6.5, 0.25 µM rsS1); skinned myofibrils at pCa50: bovine cardiac (pCa 6.25, 1 mg/mL myofibrils), human (pCa 6.0, 1 mg/mL myofibrils), bovine slow-skeletal (pCa 5.5, 1 mg/mL myofibrils), and rabbit fast-skeletal (pCa 6.0, 0.25 mg/mL myofibrils).

Data are mean ± SD of apparent IC_{50} (in µM). ND denotes the value was not determined.

The cardiac specificity of MYK-461's action was demonstrated against other myosin isoforms, fast-twitch (rabbit psoas), slow-twitch (bovine masseter) skeletal muscle, and

smooth-muscle (chicken gizzard) systems of varying complexity. Across all the systems tested, MYK-461 markedly reduced enzymatic activity (ATPase) against fast-twitch skeletal isoforms with IC50 values ranging from 4.71 µM (basal) and 5.85 µM (acto-myosin) to 2.14 µM (Ca^{2+}-dependent myofibrils) for rabbit psoas muscle. As a result of slow-skeletal muscle sharing myosin isoform β with cardiac muscle, MYK-461 inhibited slow-skeletal bovine masseter myofibrils with equal potency (IC50, 0.43 µM) as with bovine cardiac myofibrils. The applicant contends that this inhibition does not have functional (skeletal) effects in vivo (see Secondary Pharmacology, Section 4.2), and is likely due to the complete recruitability of myosins from the super-relaxed state in skeletal muscle. MYK-461 had no activity (IC50 > 50 µM) in smooth-muscle myosin-S1 in either basal or acto-myosin systems (Table 2).

The activity of MYK-461 on human cardiac myosin was slightly lower than that of bovine cardiac myosin. It was confirmed using recombinant myosin-S1 in the basal (IC50, 0.52 µM), acto-myosin (IC50, 0.73 µM), and reconstituted Ca^{2+}-regulated soluble thin-filament systems (IC50, 0.53 µM), as well as in cardiac myofibrils (IC50, 0.71 µM) (Table 2, Fig. 1)). MYK-461 also inhibited ATPase rates with high efficacy in cardiac myofibrils from various species. Incidentally, it is slightly less potent in human cardiac myofibrils than is in rodents, canine and porcine (Table 3).

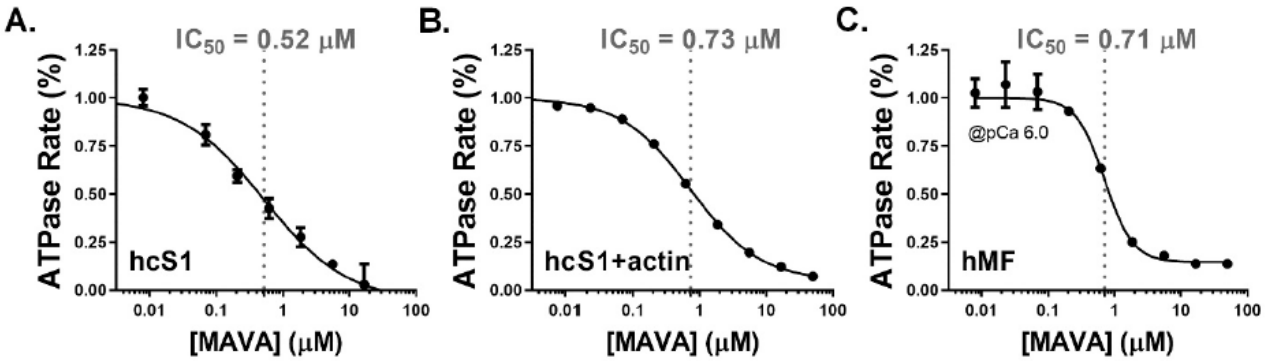


Figure 1. Inhibitory effects of mavacamten on the enzymatic activity (ATPase) of human myosin in systems of varying complexity
Mavacamten inhibited the steady-state enzymatic (ATP turnover) activity of human cardiac (beta) myosin-S1 (hcS1) in basal (motor alone) (Panel A), and actin-activated subfragment-1 systems (Panel B) as well as in ventricular myofibrils (hMF, Panel C).
Conditions: basal (myosin-S1 alone): 0.1 µM hcS1; acto-myosin (myosin-S1 + 14 µM actin): 0.25 µM hcS1; skinned myofibrils: pCa 6.0, 1 mg/mL myofibrils. pCa: activating Ca2+ concentration.
Data are mean ± SD of apparent IC50 (in µM).

MYK-461 was profiled against recombinant human beta cardiac myosin and five hypertrophic cardiomyopathy (HCM) mutant myosins. MYK-461 inhibited wild type and 5 HCM mutant forms with comparable potencies (Table 4). The IC50 values suggest that introduced mutations do not impact the inhibitory effect of MYK-461.

Table 3. Half-maximal inhibitory concentrations (IC50 mean, µM ± SD) of MYK-461 on the enzymatic activity (ATPase) in cardiac myofibrils from various species.

Species	Mavacamten IC_{50} (µM)
Human	0.71 ± 0.060
Bovine	0.49 ± 0.026
Canine	0.45 ± 0.074
Porcine	0.49 ± 0.047
Rabbit	0.76 ± 0.037
Rat	0.32 ± 0.023
Mouse	0.29 ± 0.030

Values are the calculated IC_{50} for the respective systems.

Table 4. Summary of actomyosin dose responses on recombinant human cardiac myosin (apparent IC50, mean, µM ± SD)

Protein	IC50 (µM)
wild type	0.912 ± 0.0474
R453C	0.710 ± 0.0146
R403Q	1.01 ± 0.0199
R719W	1.31 ± 0.506
R723G	1.04 ± 0.0615
G741R	0.653 ± 0.0329

MYK-461 inhibition of myosin was both noncompetitive for actin and uncompetitive with respect to ATP. Transient kinetic experiments were performed on the effect of MYK-461 on the individual kinetic steps of the myosin cycle to understand its effects on ATP binding, ADP release, phosphate (Pi) release rates of myosin, and weak-to-strong transition of myosin in the ADP-Pi state of binding to actin. MYK-461 did not affect either the kinetics of ATP binding to myosin or, once started, its hydrolysis. There appears to be slight change in this rate between the DMSO control and the presence of mavacamten, indicating that the rate of ATP hydrolysis appears unperturbed (Fig. 2 Left). Thus, there was no apparent change in the ATP binding or ATP hydrolysis rates in the presence of mavacamten.

The rate of ADP release was measured in the actin-dissociated and actin-associated states MYK-461 slowed basal ADP release (Fig. 2 Right) but preserved ADP release rates in an acto-myosin system. MYK-461 inhibited the actin-associated Pi release (Fig. 3 Left) that accompanied the power-stroke (i.e., contraction) reflecting a reduction in the number of myosin heads that are available to engage with actin in the strongly bound state. The mechanistic hallmarks of HCM are excess cross-bridge formation and

dysregulation of the super-relaxed state. MVA reduces or normalizes the probability of force-producing (systolic) and residual (diastolic) cross-bridge formation. Thus, MVA shifts the overall myosin population toward an energy-sparing super-relaxed state. The amplitudes of the weak to strong transition affected in a dose dependent manner; decreasing by 42% relative to DMSO, demonstrating that myosin S1 is being prevented from binding to actin (relaxed state) (Fig. 3 Right).

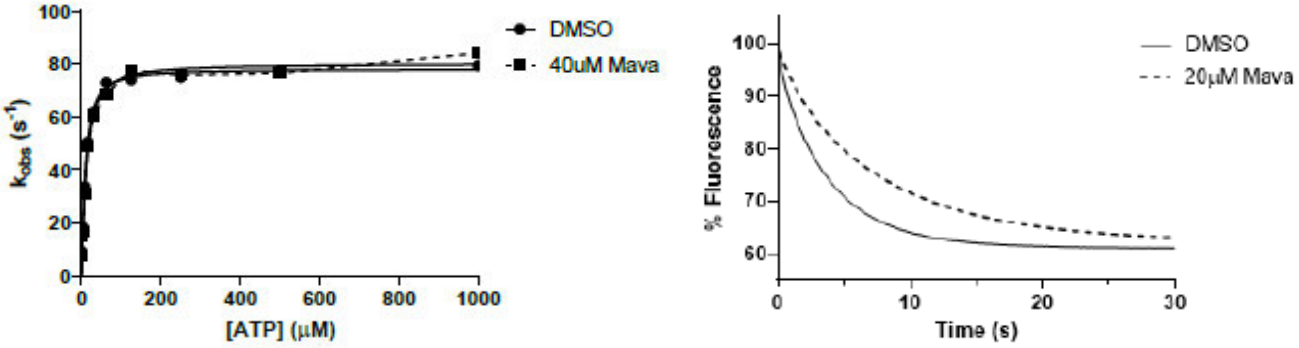


Figure 2. Transient kinetic experiments.
Left: ATP binding. The dependence of the rate of ATP binding to bovine cardiac myosin-S1 (bcS1) on the concentration of ATP. Final bcS1 concentration was 5 μM, and final ATP concentrations are those shown on the X-axis of the plot. Rates were determined by monitoring the increase of intrinsic tryptophan fluorescence of bcS1 as a function of time. Right: The effect of mavacamten on the ADP release rate from bovine cardiac myosin-S1. Basal (actin-dissociated) bovine cardiac myosin-S1 ADP release measured by mant (2′/3′-O-(N-Methylanthraniloyl))-ADP chase with ATP (1.25 μM bcS1, 1 μM mantADP, 1 mM ATP).

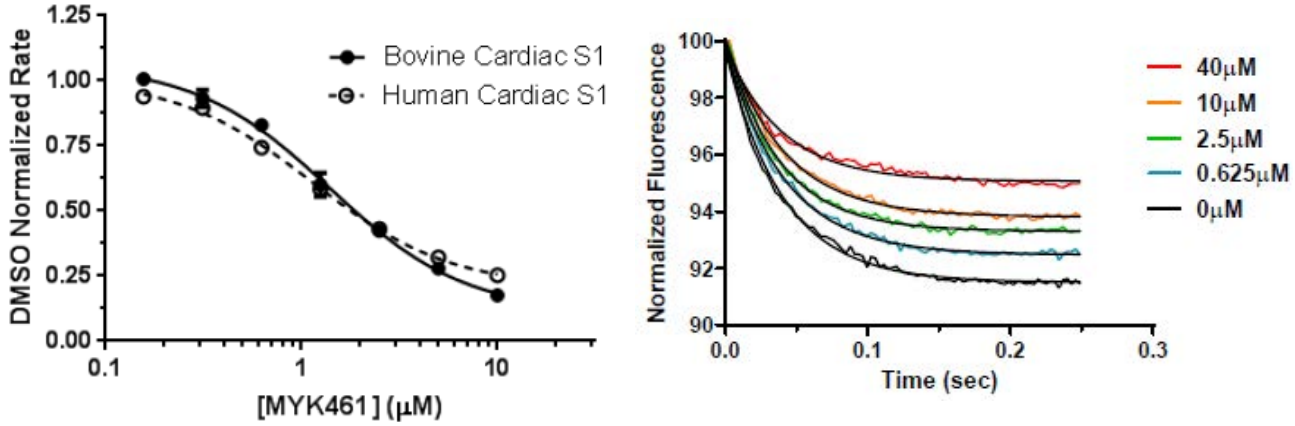


Figure 3. Transient kinetic experiments (continued).
Left: Phosphate release. Dose-response of mavacamten on bovine cardiac myosin-S1 (bcS1), measured by MDCC-PBP fluorescence in a single turnover reaction by stopped-flow. Final bcS1 concentration was 1 μM, final ATP concentration was 1 μM, and the rates were determined by monitoring the increase of MDCC fluorescence of PBP as a function of time. Right: Weak-to-Strong transition. DMSO normalized fluorescence representing the change in amplitudes with treatment with mavacamten. The effect of mavacamten on the weak-to-strong transition of bovine cardiac myosin-S1 (0.5 μM) in the ADP-Pi state binding to pyrene labeled actin (0.5 μM) and 1 mM ADP in single turnover mode.

4.1.1.2 Effects of MYK-461 In loaded and unloaded in-vitro motility assays

This non-GLP study (#NC-20-0045) was conducted at MyoKardia, Brisbane, CA (dated 11/23/20020). The objective of this biophysical study was to evaluate the effect of MYK-461 on the ability of myosin to slow actin filaments movement in both an unloaded in-vitro motility assay and a loaded in-vitro motility assay.

Methods

For in vitro motility (IVM) assay, a buffer solution containing fluorescently labeled polymerized actin filaments (tetramethyl-rhodamine phalloidin with F-actin) was applied to the bovine cardiac full-length myosin-coated surface in the presence of ATP, allowing myosin motors to cycle. Induced actin filament movement was measured by fluorescence microscopy. Dose responses on the effect of MYK-461 (0 to 40 µM) for filament velocity and length was analyzed.

For loaded in vitro motility assay (LIMA), utrophin, a strong actin-binding protein served as a frictional load to actin. The utrophin concentration (0 to 0.4 µM) is proportional to the frictional force. Since power = force × velocity, at each utrophin concentration, the power (in arbitrary units) was calculated as [utrophin] × velocity. The power curve for MYK-461 was collected at a single-concentration, at which the velocity was inhibited by 20% relative to the DMSO control (IC20), which was 62.5 ± 12.5 nM.

Results

In IVM assay, MYK-461 dose-dependently inhibited/reduced actin gliding velocities with an IC50 of 0.24 ± 0.05 µM (Fig. 4A) This effect was proportional to preventing the reduction of actin filament lengths (or increasing the actin filament length) with increasing concentrations of MYK-461 (EC50 = 0.14 ± 0.05 µM) (Fig. 4B). The effect of MYK-461 on the velocity (decreasing) and length (increasing) of actin filament is suggestive of reduced cross-bridge mediated stresses on the actin filament.

In LIMA, the utrophin concentration was proportional to the frictional force. Power output induced by utrophin was reduced by MYK-461 (at a single concentration, IC20 = 62.5 ± 12.5 nM) (Fig. 5). Reduced power resulted in a reduction in the number of cross-bridge attachments or fewer active cross-bridges.

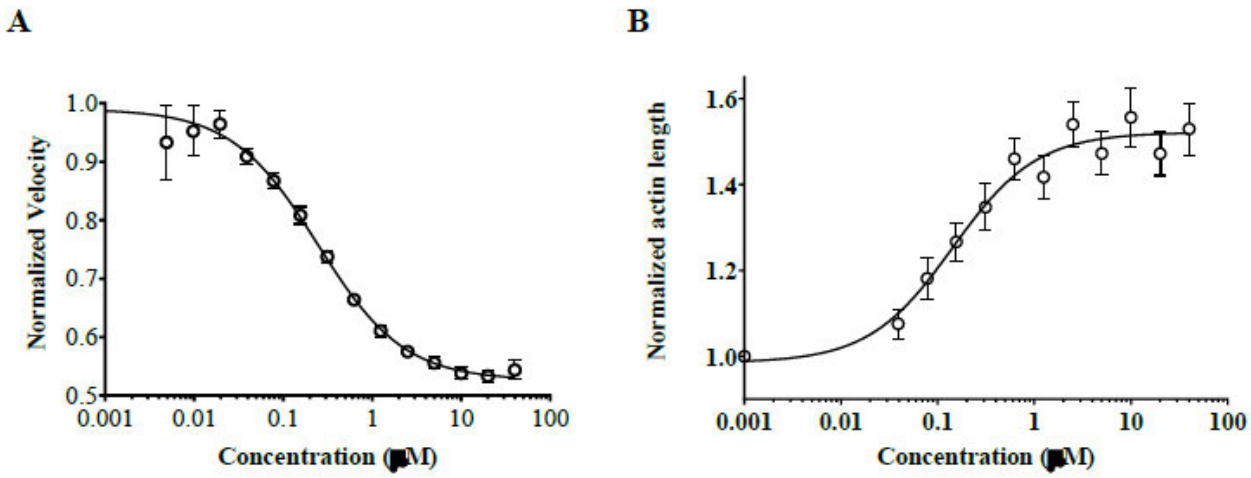


Figure 4. Dose-Response of MYK-461 in the in-Vitro Motility Assay.
Using reconstituted synthetic thick filaments (made from full-length bovine cardiac myosin), MYK-461 A) reduced actin gliding velocities with an IC50 of 0.24 ± 0.05 μM, and B) increased the actin filament length with an EC50 = 0.14 ± 0.05 μM. Data are expressed as Mean ± SEM (N=3).

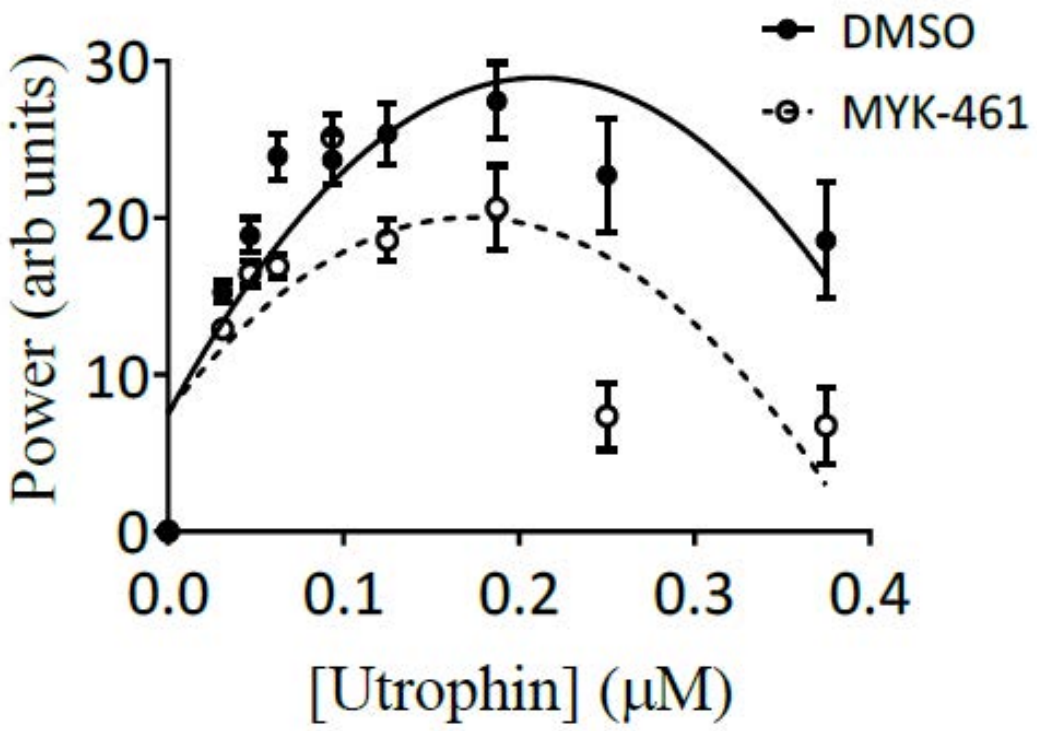


Figure 5. Loaded in-vitro motility assay.
Power curve was generated at varying concentrations of utrophin in the presence of 2% DMSO (control, solid circle) and at an IC20 (62.5 ± 12.5 μM) concentration of MYK-461 (open circle). The total power reduction by MYK-461 (area under the curve) is 38 ± 7%. Data are expressed as Mean ± SEM (N=4).

4.1.1.3 Effects of MYK-461 on the super-relaxed state of cardiac myosin

This non-GLP study (#NC-20-0046) was conducted at MyoKardia, Brisbane, CA (dated 11/24/20020). The objective of the study was to relate the biochemical mechanism of action of MYK-461 to its pharmacological effect in stabilizing the myosin heads in the super relaxed (SRX) state. Myosin exists in 3 functional states a) < 1s, ATP turnover time, b) < 10s, DRX state, c) > 100s SRX state. Thus, the objective was to evaluate the effect of MYK-461 on the distribution of myosin population among the disordered-relaxed (DRX) state and SRX state of myosin using reconstituted synthetic thick filaments (STFs) made of bovine and porcine full-length cardiac myosin.

Methods

Synthetic thick filaments (STFs) were generated from bovine and porcine cardiac full-length myosin when the ionic strength of the buffer was reduced below 150 mM.

Following incubation of myosin preparations with 2′/3′-O-(N-Methylanthraniloyl) (mant)-ATP and chased with excess unlabeled ATP, the release rates of fluorescent nucleotides (inorganic phosphate (Pi) and mant-ADP) were measured. Studies were performed in either DMSO or MYK-461 (0 to 50 μM). Fluorescence decay profiles showed two distinct phases, an early fast phase (corresponding to myosin heads readily available to interact with actin (and therefore, with intrinsically faster ATP turnover rates)), and a late slow phase (associated with a “reserve” population of myosin heads with ultraslow ATP turnover rates) corresponding to the activity of myosin heads in the DRX and SRX states, respectively.

Besides ATP chase experiments, fluorescence decay profiles were also generated using ADP chase in the presence of increasing concentrations of MYK-461. This experiment was carried out in the same way as the ATP chase experiment except that non-fluorescent ADP was used in the place of non-fluorescent ATP to chase mant-nucleotides in the second step.

Results

MYK-461 induced a concentration-dependent rightward shift of the fluorescent decay (Fig. 5A) indicating a slower release or decreased ATP turnover from myosin. In a dose-dependent manner, it shifted the overall enzymatic activity of myosin heads from the DRX state (e.g., in bovine, IC50: 0.40 μM, Fig. 5 B) toward the SRX state, suggesting a progressive reduction in the number of myosin heads readily available to form cross-bridges. MYK-461 stabilized (increased) the population of myosin heads in an energy-

sparing SRX state (reduced ATP consumption), consequently decreasing the myosin DRX population by the same amount (Fig. 5C). The potency (EC50) with which MYK-461 induces myosin into the SRX state is similar in reconstructed synthetic thick filaments from porcine (1.9 µM) and bovine (1.2 µM) full-length myosin. This increase in myosin SRX population also coincided with a concentration dependent decrease in the DRX ATPase rate with IC50 values of 0.3 and 0.4 µM in the respective systems. The SRX effects of MYK-461 were partially reversible, as myosin-heads could be recruited into the DRX state by addition of both ADP and calcium in the presence of MYK-461 (at 2 µM).

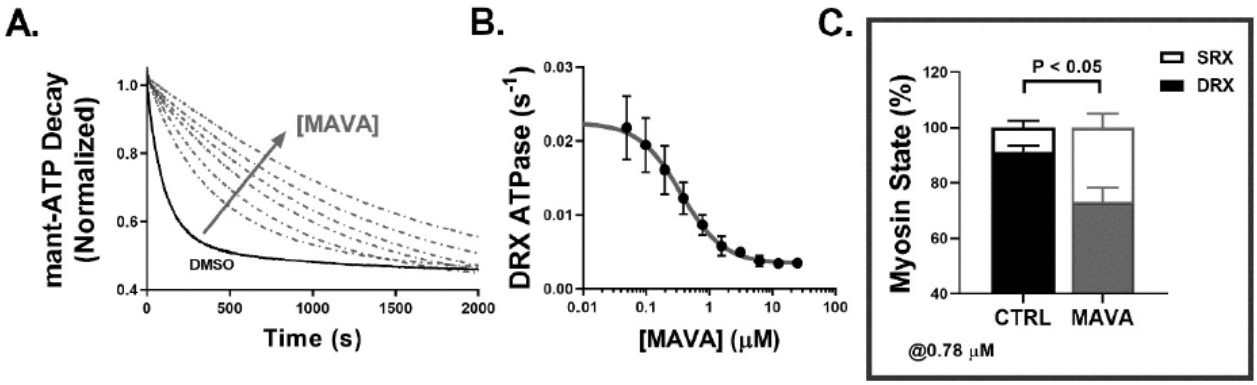


Figure 6. Effects of MYK-461 on the DRX enzymatic activity (ATPase) and the myosin states of reconstituted myosin thick filaments

DRX: disordered-relaxed; IC50: half-maximal inhibitory concentration; MAVA: mavacamten; SRX: super-relaxed.

Panel A: Representative single mant-ATP decay (following a nonfluorescent, ATP chase) in the absence (DMSO) or presence of mavacamten (diagonal arrow mark, at increasing concentrations).

Panel B: Mavacamten dose-dependently decreased the activity of the DRX myosin heads, which are otherwise readily available to interact with actin.

Panel C: Mavacamten shifted myosin heads from the DRX state toward the SRX state, favoring energy sparing (reduced ATP consumption). Data, collected using reconstituted bovine myosin thick filaments (full-length myosin), are expressed as mean ± SEM.

4.1.1.4 Effects of MYK-461 on the Pi release and ATPase rates of cardiac myosin

This non-GLP study (#NC-20-0050) was conducted at MyoKardia, Brisbane, CA (dated 11/24/20020). The study evaluated the effect of MYK-461 on the release of inorganic phosphate, the rate limiting step of the myosin ATPase cycle, and ATPase activity of actin-activated bovine cardiac myosin under both the steady-state and transient kinetic conditions. The study helps in an understanding on the overall cycling behavior of myosin in the presence of MYK-461

Methods

Steady-state ATPase assay was carried out with purified bovine cardiac myosin sub-fragment-1 (myosin-S1) and bovine cardiac actin. ATPase activity measurements were conducted with varying concentrations of MYK-461. Transient kinetic experiments were performed to determine the effects of MYK-461 on myosin rates of phosphate release.

Results

In the steady-state ATPase assay, MYK-461 dose-dependently inhibited the actin-activated myosin ATPase activity with an IC50 of 0.47 ± 0.14 µM (Fig. 7). In the transient kinetic experiments, MYK-461 inhibited the actin-associated phosphate release rate in a concentration-dependent manner with a maximal inhibition of 95% noted at the highest concentration tested (10 µM) and with an IC50 value of 0.46 ± 0.15 µM (Fig. 8A). These values are consistent with the IC50 values obtained in an actin-activated ATPase assay. The observation that MYK-461 slowed steady-state ATPase rate correlated with the single turnover Pi release rate (Fig. 8B) suggest the primary mechanism of action for MYK-461 is the inhibition of the phosphate release step in the chemo-mechanical cycle.

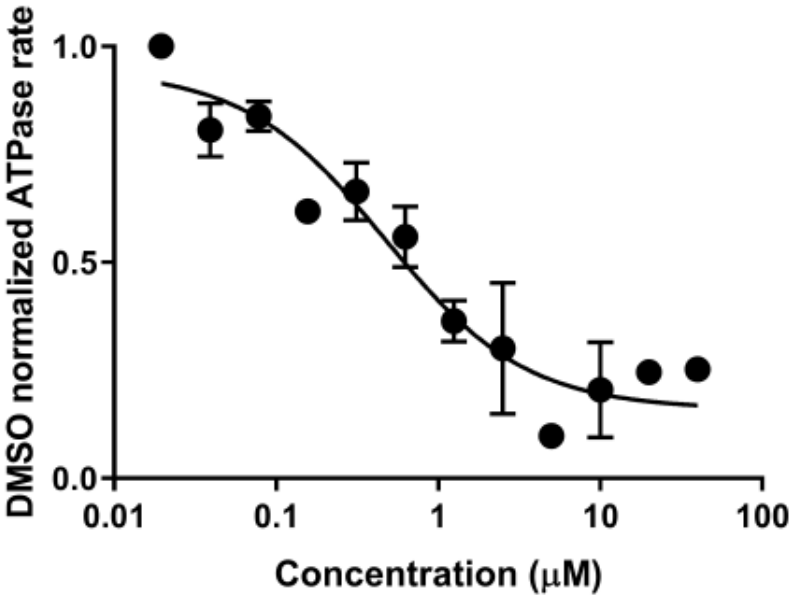


Figure 7. Effect of MYK-461 in the steady-state ATPase activity, Mean ± SEM

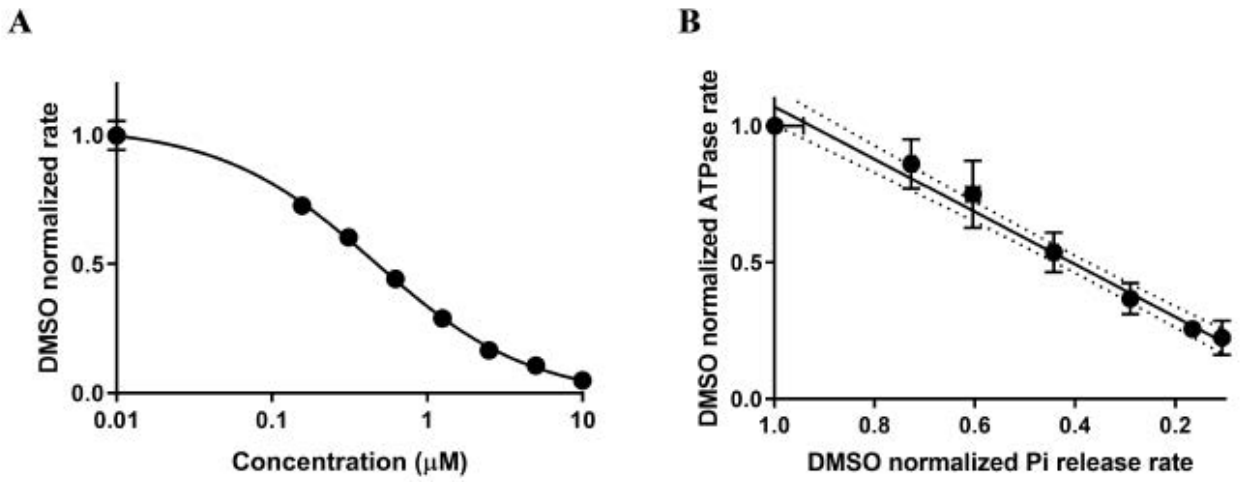


Figure 8. Effect of MYK-461 in the transient kinetic actin-activated phosphate release.
A: IC50 = 0.46 ± 0.15 μM. Data are expressed as Mean ± SEM (n = 3). B: Reduction in Pi release rate linearly predicts the decrease in steady state ATPase rate due to increasing MYK-461 concentration (n = 2; Pearson's R2 = 0.98, P<0.05).

4.1.2. *Ex vivo* studies

4.1.2.1 Effects of MYK-461 on the Pi release and ATPase rates of cardiac myosin

This non-GLP study (#NC-14-0006) was conducted at MyoKardia, Brisbane, CA (dated 11/24/20020). The effect of varying concentrations of MYK-461 was evaluated in adult rat ventricular myocytes on the cellular contractility and calcium cycling for cellular fractional shortening (FS).

Methods

Cardiac myocytes were prepared from male Sprague Dawley rats (body weight, 300 to 350 g). After an initial baseline period, contractility measurements were made in the presence of MYK-461 (0.03 μM to 1 μM) to determine the cellular IC50. For FS, data were normalized to basal values (basal equals 100%) and expressed as FS%. Myocyte calcium homeostasis was evaluated in the presence and absence of MYK-461 determining whether the observed decrease in contractility has resulted from modulating the calcium transient. To demonstrate that a β-agonist can restore MYK-461 induced inhibition, myocytes were treated with 0.02 μM isoproterenol in the presence of 0.25 μM MYK-461 and contractility measurements were obtained.

Results

A concentration-dependent decrease in contractility/fractional shortening (IC_{50} of 250 nM, Fig. 9) and contraction velocity (55% at 0.3 µM) was noted. A significant inhibition of contractility was observed at the highest concentration, 1 µM (Table 5). Reduced myocyte contractility is consistent with the mechanistic biochemical data suggesting MYK-461 stabilizes myosin in the "off-actin" state, reducing aggregate force production. At concentrations up to 1 µM, the cell length at diastole (resting length) remained unchanged but shortened the relaxation (re-lengthening) time, RT50: -24% at 1 µM, P < 0.05) (Table 5)

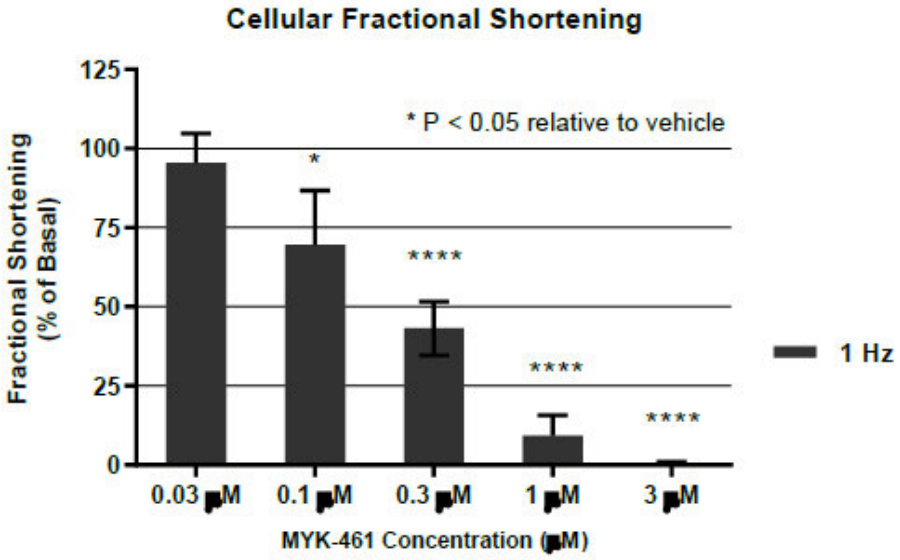


Figure 9. Effect of MYK-461 on cellular contractility in rat ventricular myocytes (Mean ± SD)

Table 5. Concentration dependent effects in the presence of MYK-461 (Average ± SD, n = 3-8 cells per concentration) in cardiac myocytes

Species/Condition (n = 8 cells/each)		Resting Length (µm)	Vshortening (µm/s)	SF (%)	RT_{50} (s)
Rat Myocytes	PRE	134 ± 3	-232 ± 29	7.2 ± 0.9	0.14 ± 0.01
	+MAVA (0.3 µM[a])	135 ± 3↑	-103 ± 12↓	3.2 ± 0.5↓	0.13 ± 0.01↓
	Change vs PRE (%)	*+0.6 ± 0.2*	*-55 ± 3*	*-57 ± 3*	*-8 ± 3*
	PRE	146 ± 4	-201 ± 21	6.3 ± 0.4	0.13 ± 0.01
	+MAVA (1.0 µM)	148 ± 4↑	-27 ± 7↓	0.6 ± 0.2↓	0.10 ± 0.00↓
	Change vs PRE (%)	*+1.2 ± 0.1*	*-87 ± 2*	*-91 ± 2*	*-24 ± 4*

PRE: predose; RT50: re-lengthening/ relaxation time, time to reach 50% of the resting length; Vshortening: shortening velocity; SF: fractional shortening during systole.
a Combined data for 0.3 (n = 4) and 0.25 µM (n = 4).
↑, ↓: P < 0.05 vs PRE.
Data are mean ± SEM, showing absolute value both pre-exposure/baseline (PRE) as well as at 5 min post-exposure (MAVA); changes (from PRE) are shown in italics.

The role of calcium in decreasing the contractility was studied by measuring calcium transients in the presence and absence of 250 nM MYK-461. The study showed that decrease in contractility (reduction in %FS) to MYK-461 is independent of calcium flux as cytosolic Ca^{2+} transients were preserved in the presence of MYK-461, confirming that, unlike traditional negative inotropes that act by modulating calcium flux, its inhibitory mechanism of action is Ca^{2+} sparing (i.e., does not affect Ca^{2+} fluxes) and, therefore, unlikely to increase proarrhythmic risks. (see also section 4.1.2.2).

Beta-adrenergic agonists, e.g., isoproterenol (2 nM) and dobutamine, counteracted the inhibitory effect of MYK-461 (40% inhibition at 250 nM) in adult rat ventricular myocytes (Fig. 10). The opposing effects of MYK-461 and isoproterenol on contractility (measured as fractional shortening) demonstrated that MYK-461 decreases the ability of 'myosin' to bind to 'actin' in the strongly-bound state (i.e., stabilizes the 'weakly-bound' or 'off-actin' state of myosin), which translates to decreased contractile force of the myocardium. On the other hand, isoproterenol stimulated the excitation-contraction coupling process in the presence of 0.25 µM MYK-461 to allow additional myosin heads to overcome the inhibitory effect imposed by MYK-461. Isoproterenol recruits 'off-actin' myosin heads to the 'on-actin' state, increasing fractional shortening. The data are summarized in Figure 10 and Table 6. Also, see the next section. A similar study was done in vivo using dobutamine instead of isoproterenol (see sections 4.1.3.4 and 4.1.3.6).

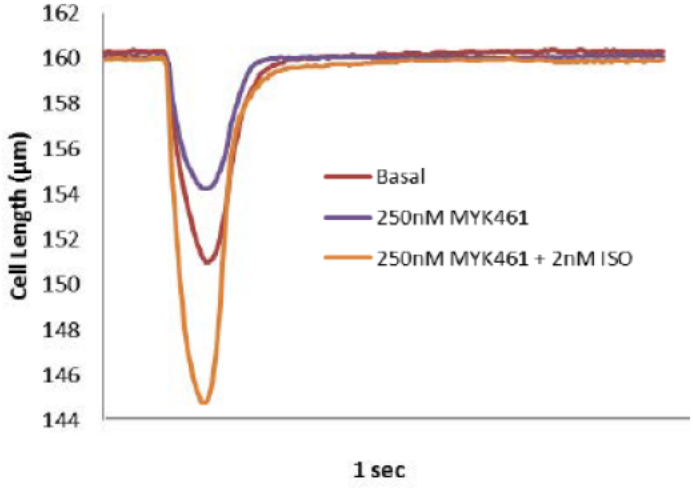


Figure 10. Isoproterenol recovery from MYK-461 modulation

Table 6. Isoproterenol countering the effect of MYK-461 (Mean ± SD)

	Cell Length (% of basal)	Fractional Shortening (% of basal)	Time to Peak (% of basal)	Contraction Velocity (% of basal)	Time to Baseline 75% (% of basal)
Basal	100	100	100	100	100
0.25µM MYK-461	100.1 +/- 0.7	59.6 +/- 5.7	92.8 +/- 4.2	67.0 +/- 19.1	96.7 +/- 8.6
0.25 µM MYK-461 + 0.02µM ISO	100.3 +/- 0.5	159.5 +/- 26.4*	91.7 +/- 1.0	187.9 +/- 25.1*	86.7 +/- 4.8

4.1.2.2 Effects of β-adrenergic receptor stimulation on MYK-461-induced functional depression of primary ventricular myocytes from healthy rats

This non-GLP study (#NC-20-0052) was conducted at MyoKardia, Brisbane, CA (dated 11/23/20020). The functional (contraction) and calcium handling (calcium-transient) effects of MYK-461 were evaluated both at rest and in the presence of β-adrenergic stimulation with isoproterenol in rat ventricular myocytes.

Methods

As detailed in the previous section, 4.1.2.1.

Cardiomyocyte calcium transient studies were made in the presence of vehicle (pretreatment), MYK-461 and isoproterenol with or without MYK-461. Relevant quantified measurements included: diastolic calcium, peak systolic calcium, calcium transient amplitude (cycling calcium), transient decay time of return to 50% from peak (RT50%, in seconds) and transient decay time of return to 75% from peak (RT75%, in seconds). Sarcomere length (SL) signals were quantified for each treatment. The measurements included diastolic (resting) SL (µm), cell shortening % (percent of contraction amplitude to diastolic SL), maximum SL contraction velocity (µm/second), SL time of return to 50% from peak systolic contraction (RT50%, in seconds) and SL time of return to 75% from peak systolic contraction (RT75%, in seconds).

Results

MYK-461 treatment at 0.25 µM applied for 5 min significantly decreased (-62 ± 7% vs. control, $P < 0.05$) cardiomyocyte cell shortening fraction, as measured by percent change in sarcomere length from resting diastolic length (Fig. 11). Addition of isoproterenol (2 nM for 3 minutes) at the peak level of inhibition, restored cell shortening (-22 ± 11% vs. control, $P > 0.05$) to some extent (Fig. 11 and 12 Left) and could not restore to pretreatment level. Nevertheless, it demonstrated the recruitability of the mavacamten-induced inhibition of contractile functioning at the cellular level. Also, MYK-461 markedly decreased (-58 ± 8% v. CTRL, $P < 0.05$) contraction velocity that was partially restored toward pretreatment levels with isoproterenol (-16 ± 13% vs. control, $P = > 0.05$) (Fig. 12 Right).

The experiment also showed that treatment with MYK-461 accelerated cardiomyocyte re-lengthening times both at the mid (RT50%: -18 ± 3% vs. control, $P < 0.05$, Fig. 13 Left) and late (RT75%: -18 ± 4% vs. CTRL, $P < 0.05$) phases following peak contraction. Treatment with isoproterenol maintained MYK-461-induced enhancement of relaxation

(lusitropy) (RT50%: -16 ± 3% vs. control, P < 0.05, RT75%: -19 ± 3% vs. control, P < 0.05). MYK-461 accelerated relaxation dynamics, wherein it increased diastolic (resting) SL (+2.2 ± 0.6% vs. CTRL, P < 0.05) and prevented the characteristic isoproterenol-induced reductions in diastolic SL (+2.6 ± 0.6%, 1.88 ± 0.01 vs. 1.83 ± 0.01 μm in control + ISO, P < 0.05) (Fig. 13 Middle). As noted in previous studies, MYK-461 preserved systolic (Fig. 13 Right) and diastolic calcium-transient amplitudes. These results lend evidence to support observations of MYK-461 to inhibit cardiomyocyte contractility while preserving the β-adrenergic response and the lusitropic effects without interfering with the calcium transient.

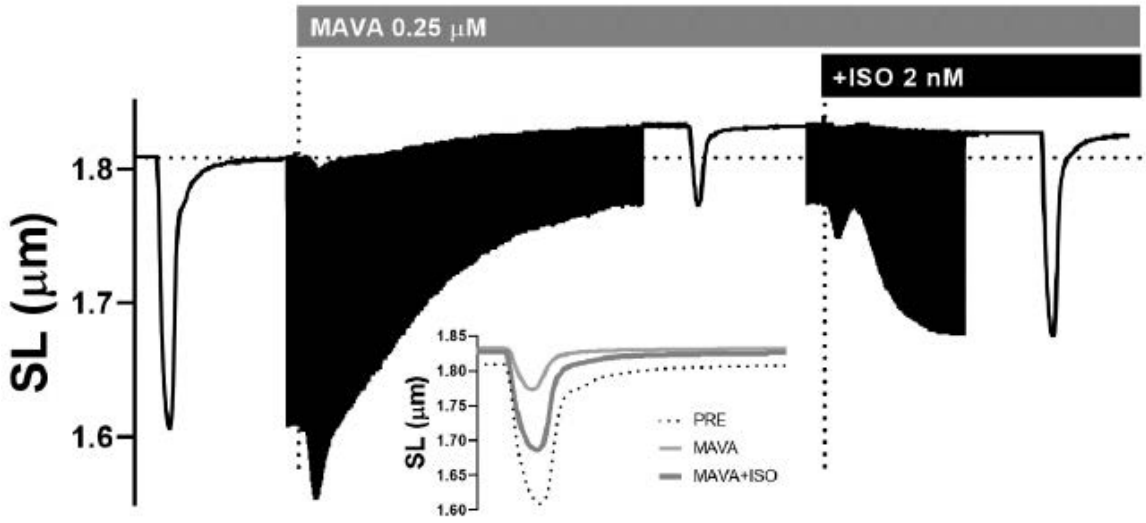


Figure 11. Effect isoproterenol (ISO) on MYK-461-induced functional depression of myocytes showing reductions in twitch amplitudes and concomitant increase in resting lengths under MYK-461 exposure as well as the functional rescue triggered by an overlapping ISO challenge.

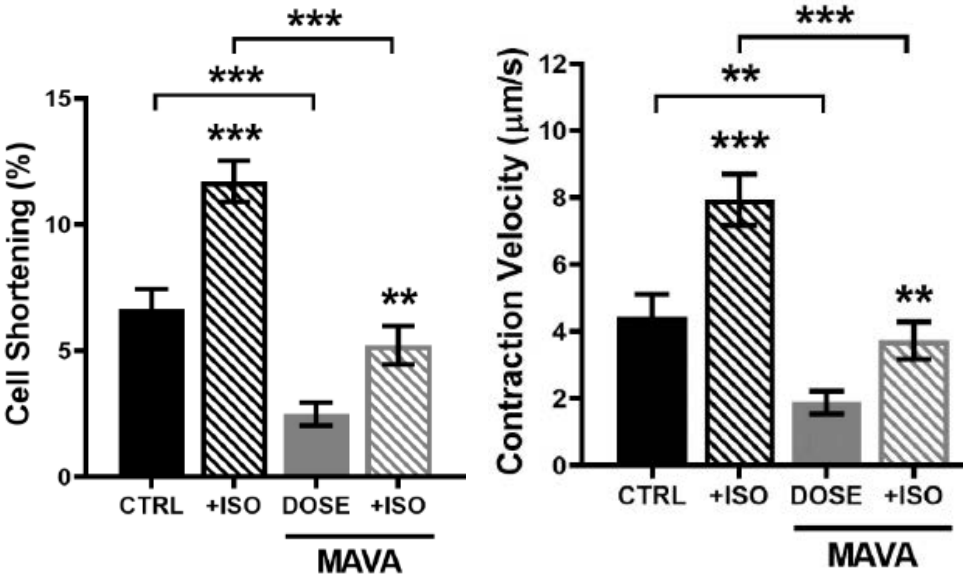


Figure 12. Effect isoproterenol (ISO) on MYK-461 reduced shortening fraction (%) and contraction velocity.

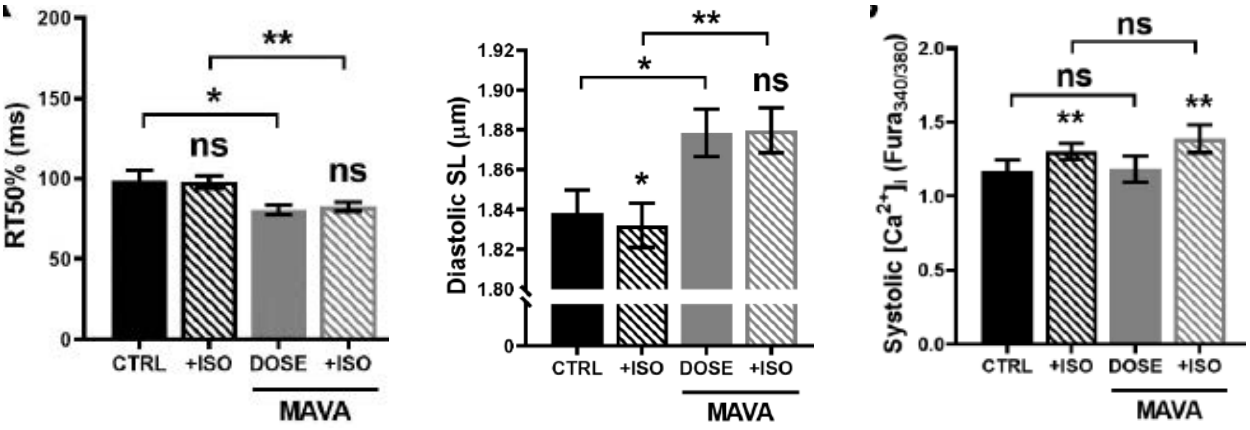


Figure 13. Effect isoproterenol (ISO) on MYK-461-induced inhibition of contractile function.
Left: MYK-461 hastened cardiomyocyte re-lengthening/relaxation times both at the mid (RT50%) and late (RT75% (not shown) phases following peak contraction. MYK-461-induced enhancement of relaxation was maintained with isoproterenol treatment while preserving intracellular Ca^{2+} transients (systoli $[Ca^{2+}]_i$ shown, Right panel) when compared with time-matched controls. Center: MYK-461 produced elongated diastolic sarcomere lengths and prevented shortening of the diastolic sarcomere length with isoproterenol challenge.

4.1.2.3 Effects of stretch and β-adrenergic receptor blockade on the cardiac effects of MYK-461 in isolated rat hearts

This non-GLP study (#NC-20-0063) was conducted at MyoKardia, Brisbane, CA (dated 12/18/20020). The cardiac functional effects (systolic and diastolic responses) of MYK-461 were evaluated both under control conditions and under β-adrenergic receptor (AR) blockade in isolated Langendorff-perfused rat hearts.

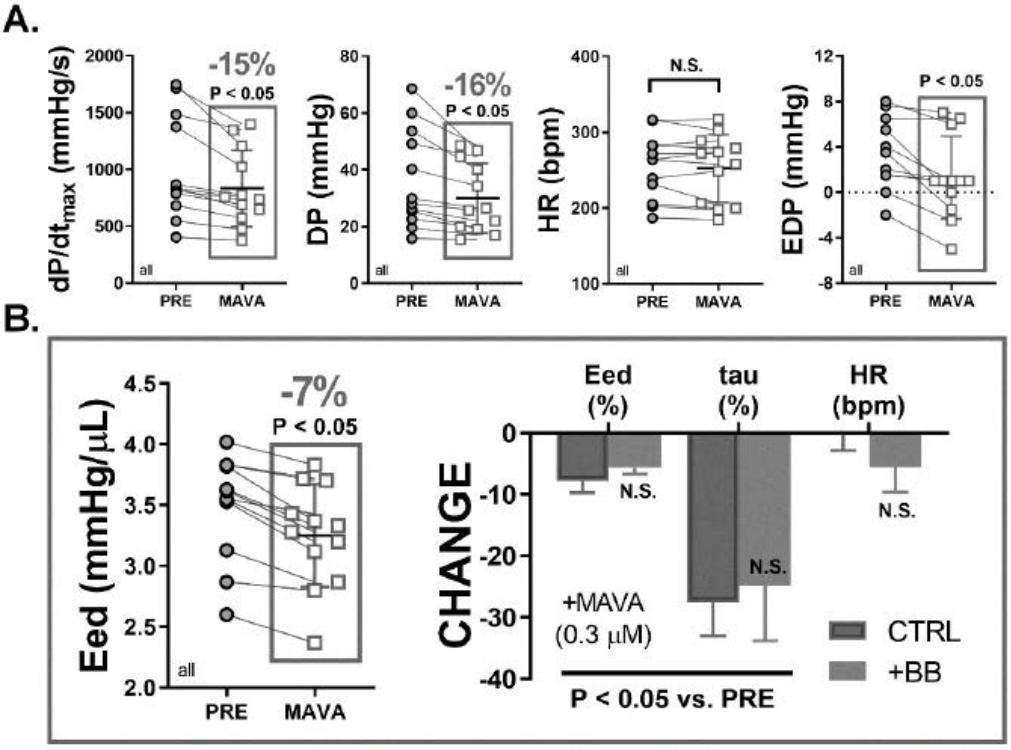
Methods

Under surgical plane of anesthesia, the heart was removed from male Sprague-Dawley rats (body weight, 300 to 450 g). The heart was placed in cold perfusate Tyrodes solution and immediately cannulated retrograde via the aorta onto a Langendorff perfusion system. The hearts were instrumented for the recording of ECG via two surface epicardial electrodes and left ventricular pressures (LVP). Left ventricular function (isometric/isovolumic) both at steady-state as well as during rapid stretch challenges were studied before and after MYK-461 administration (0.3 µM) in two sets of experiments: one under control conditions (n = 6) and the other after β-AR blockade (metoprolol 0.1 µM + 10 nM of isoproterenol; n = 7).

Results

In the isolated rat heart, MYK-461 at 0.3 µM decreased the peak-rate of systolic pressure development (dP/dtmax, -15%) and developed pressures (DP, -16%) (both

indicative of negative inotropy), while reducing EDP (1 vs.3 mm Hg, P < 0.05) and improving end-diastolic stiffness (Eed: -7%, P < 0.05). Furthermore, MYK-461 hastened relaxation by shortening the time-constant of relaxation (6.4 vs. 8.3 ms, P < 0.06, -22%). (Fig. 14). These effects were observed free of any concomitant chronotropic effects. The heart was responsive to isoproterenol-induced inotropic and chronotropic agonism (dP/dtmax: 36 ± 6% and HR: 17 ± 3%, both P < 0.05). Addition of metoprolol in the presence of isoproterenol did not affect the cardiac negative inotropy with concomitant positive lusitropy of MYK-461. MYK-461 still decreased DP (-10%) and dP/dtmax (-11%) while lowering EDP (-1 mm Hg). The pro-compliant and lusitropic effects of mavacamten under β-AR blockade (Eed: -6% and tau: -31%, both P < 0.05) were similar to those observed under control conditions. In summary, direct myosin attenuation with MYK-461 has a unique profile characterized by negative inotropy with improved left ventricular compliance. This novel profile was preserved in the presence of β-adrenergic blockade.



β-AR: β-adrenoreceptor; CTRL: control; dP/dtmax: peak rate of left ventricular pressure increase during systole; PRE: predose.
Panel A: In isolated rat hearts, mavacamten (MAVA, 0.3 µM) decreased dP/dt_{max}, developed pressures (DP), and end-diastolic pressures at steady state (EDP), without affecting heart rate (HR); *Panel B*: mavacamten also reduced estimated stiffness (EDP changes in response to a 30-µL preload increase; Eed) and the time constant of relaxation (tau); β-AR blockade (+BB, metoprolol 0.1 µM) did not alter these responses.
Data are mean ± SEM, showing steady-state values before (PRE) and during treatment values.

Figure 14. Left ventricular effects of MYK-461 (mavacamten) in isolated perfused rat hearts

4.1.2.4 Biomechanical effects of MYK-461 in skinned ventricular muscle fibers from healthy animals

This non-GLP study (#NC-20-0048) was conducted at MyoKardia, Brisbane, CA (dated 11/23/20020). The effect of MYK-461 on tension development and stiffness under mechanical stress (length dependence) was evaluated in skinned ventricular papillary muscle fibers from rats and pigs. In addition, the effects of MYK-461 were evaluated under stretched conditions to resolve the state of myosin, the super-relaxed state (SRX) and disordered-relaxed state (DRX) utilizing MANT-ATP dissociation kinetics. Skinned muscle fibers are a permeabilized muscle assay designed to test muscle function in the absence of cell membranes and Ca^{2+} handling machinery. It preserves the architecture of the cardiac sarcomere, allowing for measurement of force generation in response to Ca^{2+}.

Methods

Left ventricular papillary muscle fiber bundles from the hearts of male Sprague-Dawley rats and male Yucatan mini-pigs were rapidly dissected and cut to approximately 4 mm x 200 µm. The fibers were chemically skinned in high relax solution (100 mM BES, 10 mM EGTA, 6.57 mM MgCl2, 6.22 mM ATP, 5 mM NaN3, 10 mM creatine phosphate, 5 U/ml creatine phosphokinase, adjusted to 180 mM ionic strength with 41.89 mM K-propionate, pH 7.0) containing 1% Triton X-100 prior to fitting with aluminum T-clips for force and mechanical measures. Force was generated by exposing single muscle fibers dissected from larger segments of tissue to increasing concentrations of calcium. For each muscle fiber, force/pCa relationships were first measured in the absence (1% DMSO) and presence of MYK-461 (dissolved in 1% DMSO). For length dependence experiments, individual fibers were tested for the effects of stretch (i.e., sarcomere length 2.0 stretched to 2.3 µm) in the absence (1% DMSO) and presence of MYK-461 (in 1% DMSO). The role of the SRX of myosin on length dependent activation was investigated by measuring the rate of MANT-ATP [(2'-(or-3')-(O-(N-Methylanthraniloyl Adenosine 5'-Triphosphate] dissociation from pig skinned myocardial fibers.

Results

MYK-461 dose-dependently decreased the tension produced at each calcium concentration (Fig. 15A), effectively reducing maximal tension/force (e.g., at 1 µM, 41.2 mN/mm2 in control to 19.4 mN/mm2 (19% from basal) when treated, $P < 0.05$) without affecting intrinsic thin filament properties such as Ca^{2+} sensitivity (pCa50) or Ca^{2+} cooperativity (Hill slope). Similar observations were made in porcine skinned fibers

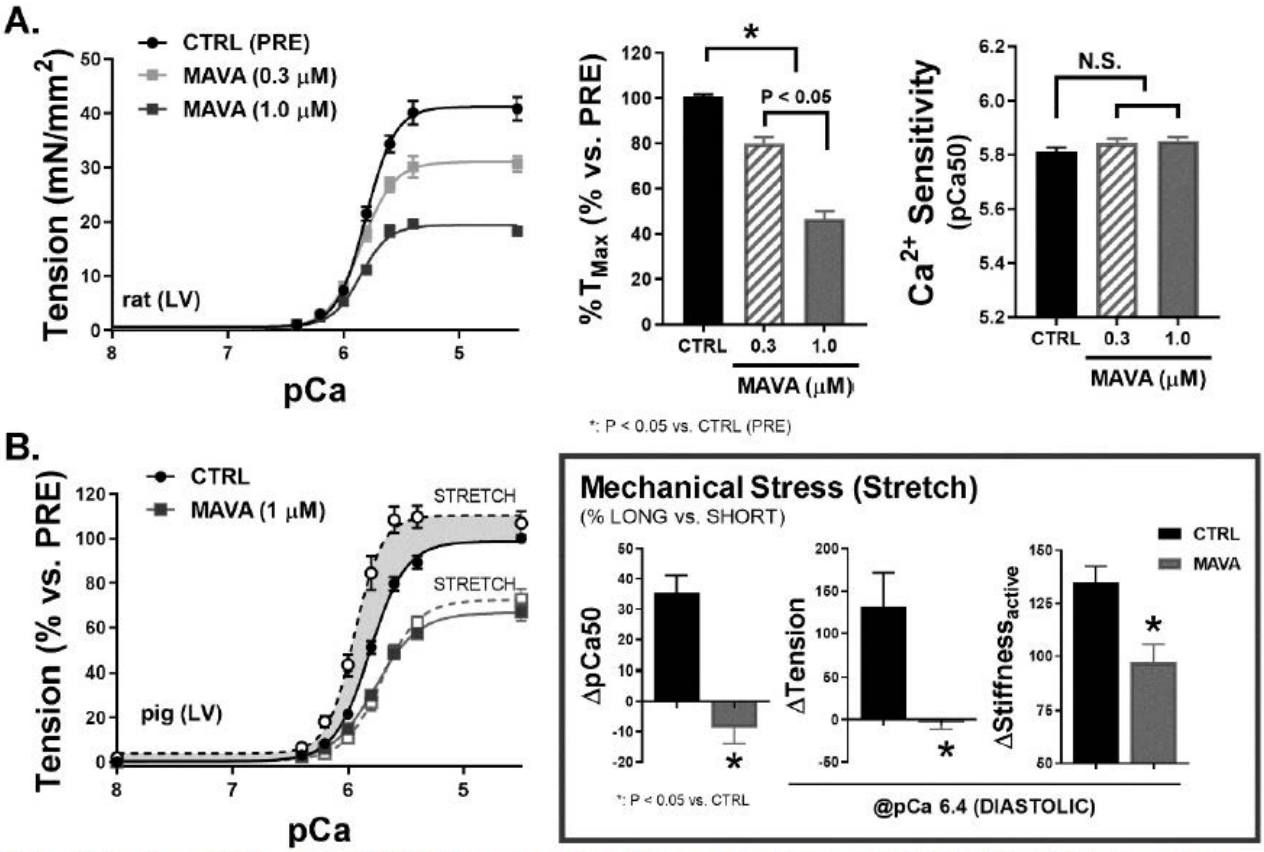
(Table 7). This profile is consistent with a mechanism that removes myosin heads from actin engagement, thus reducing force production at a given Ca^{2+} concentration.

Table 7. Effects of MYK-461 on isometric tension development in skinned ventricular fibers from rat and pig at varying calcium concentrations

Species/Condition (at 2.0 µm SL)		Maximal Tension (T_{max})		pCa50	Hill Slope
		mN/mm^2	%	n/u	n_H
Rat	PRE	41.2 ± 1.1	100.8 ± 1.4	5.81 ± 0.01	3.5 ± 0.2
	MAVA (0.3 µM)	31.1 ± 0.9*	80.0 ± 1.8*	5.84 ± 0.02	3.1 ± 0.3
	MAVA (1.0 µM)	19.4 ± 0.5*	46.6 ± 1.6*	5.85 ± 0.02	3.5 ± 0.6
Pig	PRE	24.7 ± 0.1.2	100.0 ± 4.5	5.82 ± 0.01	3.4 ± 0.3
	MAVA (1.0 µM)	17.9 ± 1.8*	72.3 ± 7.3*	5.79 ± 0.02	2.9 ± 0.2

MAVA: mavacamten; pCa50: [Ca^{2+}] at 50% of maximal activation; PRE: predose; SL: sarcomere length; T_{max}: maximal isometric tension (may not necessarily occur at pCa 4.5, the highest tested).
*: $P < 0.05$ vs PRE.
Data are mean ± SEM, both pre-exposure/baseline (PRE) as well as at steady-state post-exposure (MAVA).

In response to stretch (mechanical stress by increasing sarcomere length from 2.0 µm to 2.3 µm), skinned porcine muscle preparations showed a left-upward shift in the tension/pCa relationship (Fig. 15B, left) with increased parameters of contractility. Stretch was associated with robust increases in maximal force (99.3 to 111.8%, $P < 0.05$), Ca^{2+} sensitivity (pCa50: 5.80 to 5.93, $P < 0.05$), and Ca^{2+} cooperativity (Hill slope, 2.86 to 3.65, $P < 0.05$). Stretch also increased active stiffness at both systolic and pre-activating/diastolic calcium levels (pCa 5.8 and 6.4, respectively) and accelerated the MANT-ATP decay (at relaxed Ca^{2+}, pCa 8.0), suggesting increased availability of myosin heads (and higher ATP use) with stretch. Mechanical stress increased pre-activating tension (at pCa 6.4), a potential surrogate for end-diastolic tension in vivo. MYK-461 (at 1 µM) blunted the effects of stretch, preventing sarcomere activation (sensitization) and diastolic tension development/stiffening (at pCa 6.4: -3.1 vs 131.4% in control, $P < 0.05$) (Fig. 15B, right box) due to mechanical stress while slowing MANT-ATP decay (diastolic ATP consumption). These observations are consistent with a mechanism that mavacamten-induced inhibition of myosin and resulting reduction in the number of myosin heads entering force production states effectively decreases the probability of cross-bridge formation and translates to reductions in myofilament tension generation at both systolic and diastolic Ca^{2+} levels.



Ca^{2+}: calcium ion; CTRL: control; MAVA: mavacamten; N.S.: not significant; pCa50: [Ca^{2+}] at 50% of maximal activation; PRE: predose; STRETCH: mechanical stretch between 2.0 (SHORT) and 2.3 μm (LONG) sarcomere lengths.

*: $P < 0.05$ vs CTRL (pretreatment).

Panel A: In skinned rat ventricular (papillary) muscle fibers, mavacamten dose-dependently decreased maximal isometric tension (T_{Max}) while preserving Ca^{2+} sensitivity (pCa50) and/or cooperativity. *Panel B*: In skinned pig ventricular (papillary) muscle fibers, mavacamten (at 1 μM) decreased T_{Max} and blunted the fiber response to mechanical stress (stretch), preventing sarcomere activation/sensitization and diastolic tension development/stiffening.

Data are mean ± SEM, at pre-exposure/control-condition (CTRL/PRE) and at steady-state post-exposure (MAVA).

Figure 15. Biomechanical effects of MYK-461 in skinned ventricular fibers: blunted response to mechanical stress (stretch)

4.1.2.5 Biomechanical effects of MYK-461 in skinned ventricular muscle fibers with pathogenic HCM mutations

This non-GLP study (#NC-20-0064) was conducted at MyoKardia, Brisbane, CA (dated 12/18/20020). The biomechanical effects of MYK-461 were evaluated on skinned left ventricular papillary muscle fibers from pigs carrying three different mutations in sarcomeric proteins known to be pathogenic for hypertrophic cardiomyopathy (HCM) in humans: 1) the R403Q point mutation in beta-myosin (MYH7 gene), 2) a truncating mutation (E330X) in cardiac myosin binding protein C (MYBPC3 gene), and 3) the R193H point mutation in cardiac troponin I (TNNI3 gene). Skinned muscle fibers are a permeabilized muscle designed to test muscle function in the absence of cell membranes and Ca^{2+} handling machinery. It preserves the architecture of the cardiac sarcomere, allowing for measurement of force generation in response to Ca^{2+}. Also, the ability of MYK-461 to normalize (reduce) the pool of myosin existing in a disordered-relaxed state (DRX) ready to interact with actin, by stabilizing the SRX state, were tested in LV tissues from R403Q MYH7 mutant pigs; this mutation has been shown to alter cross-bridge kinetics by freeing (or dysregulating) excess myosin-heads from the SRX "reserve" pool.

Methods

For the first study, left ventricular papillary muscle fiber bundles from the hearts of male Yucatan mini pigs were prepared and setup for force and mechanical measures as described in the previous section. All muscle fibers were tested against a range of calcium in the untreated state followed by the treated state to determine the effect of MYK-461 on each individual fiber.
For the second study, cardiac muscle tissue homogenates were prepared from left ventricular muscle of 9-month-old WT and HCM-causing R403Q mutant pig lines. Following the homogenization, the muscle samples were centrifuged and were subjected to detergent skinning overnight. After centrifugation, the sample was suspended and incubated with 2′/3′-O-(N-Methylanthraniloyl) (mant)-ATP and chased with excess unlabeled ATP. During the single turnover chase phase, fluorescence decay profiles (an early fast phase and a late slow phase) were acquired using varying concentrations of MYK-461, ranging from 0 to 50 μM.

Results

In all skinned LV fibers (WT and 3 mutations), MYK-461 decreased the tension produced at each calcium concentration (pCa), reducing maximal, sub-maximal (pCa

6.0), and diastolic tensions, effectively blunting the effects of the mutations (Fig. 16). MYK-461 also normalized Ca^{2+} sensitivity. In ventricular myofibrils from both wildtype and HCM pigs (R403Q MYH7 mutant), MYK-461 increased, and in disease restored, the population of myosin heads in the SRX state in ventricular muscle fibers, restoring the dysregulated (reduced) SRX population in HCM toward normal (Fig. 17).

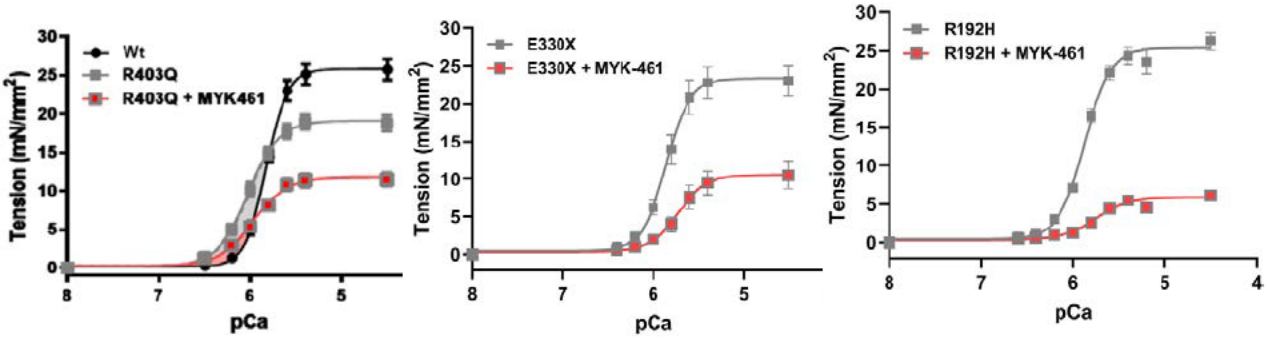


Figure 16. Tension/pCa curves for skinned ventricular fibers with the WT and the mutant (R403Q, E330X, R192H) in the absence (gray) and presence of MYK-461 (1 µM, red).

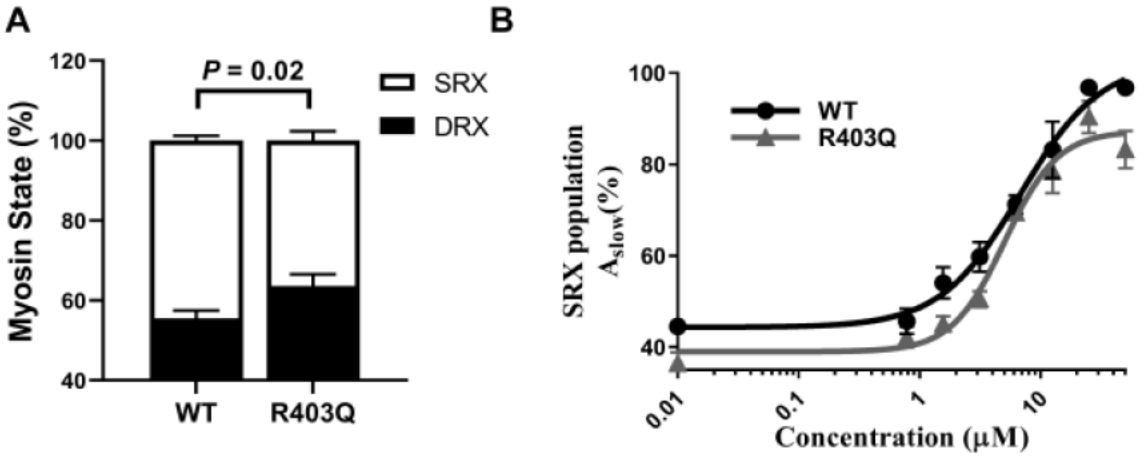


Figure 17. Myosin States in Wild-Type (WT) and HCM-Mutant (R403Q) Left-Ventricular Muscle (A), and the Effects of MYK-461 on the SRX population (B).
Panel A: Overall myosin populations in the DRX and SRX states.
Panel B: Myosin SRX population as a function of MYK-461 concentration. Data are expressed as mean ± SEM (n = 12 from three hearts).

4.1.2.6 Functional effects of MYK-461 on human induced pluripotent stem cell derived cardiomyocytes

This non-GLP study (#NC-20-0049) was conducted at MyoKardia, Brisbane, CA (dated 11/24/20020). The study examined the effects of MYK-461 on the contractility of human induced pluripotent stem cell derived cardiomyocytes. Contractility was demonstrated via two independent measures, impedance and imaging-based sarcomere tracking (SarcTrac).

Methods

Human iPSC-cardiomyocytes were obtained from two healthy volunteers. Contractility was assessed using changes in monolayer impedance amplitude recorded with a Cardio instrument. Following baseline measurements, CMs were treated with MYK-461 at 0.1, 0.2, 0.5, 1, and 2 µM in quadruplicate, in 0.25% DMSO as a vehicle. Following treatment, impedance was recorded for 20s every 10 minutes starting at 5 minutes, for a period of 90 minutes. Vehicle- and MYK-461-treated iPSC-CMs were then electrically paced at 1.5 Hz (90 beats/minute) to assess their contractile properties.

Results

MYK-461 decreased contraction amplitude in a dose- (IC50, 0.204 µM) and time-dependent manner. It decreased contraction amplitude within 5 min of application at concentrations higher than 0.5 µM. The maximal effect for all applied concentrations was achieved within 20 minutes (Fig. 18 A, B, D). MYK-461 significantly decreased the time to peak (CT100, Time to 100% of maximum contraction time), RT50 (relaxation time to 50% of maximum relaxation time), RT slope, and IBD50 (Intermediate Beat Duration from CT50 to RT50), relative to vehicle in both spontaneously-beating and paced CMs. At the same time, MYK-461 increased beating rate of treated CMs at all concentrations tested (up to 1 µM) (Fig. 18C) except at 2 µM as a result of complete inhibition of contraction. Besides contractility, MYK-461 markedly and dose-dependently reduced (IC50 = 0.1 µM) the degree of sarcomere shortening during both contraction and relaxation of CMs. Also, it increased the sarcomere length of CMs at steady state during systole (contracted sarcomere length) (Fig. 19 Left) and diastole (relaxed sarcomere length) (Fig. 19 Right) relative to vehicle. Furthermore, MYK-461 decreased the duration of contraction (time to peak contraction after peak relaxation) and hastened relaxation (time to peak relaxation after peak contraction) transients.

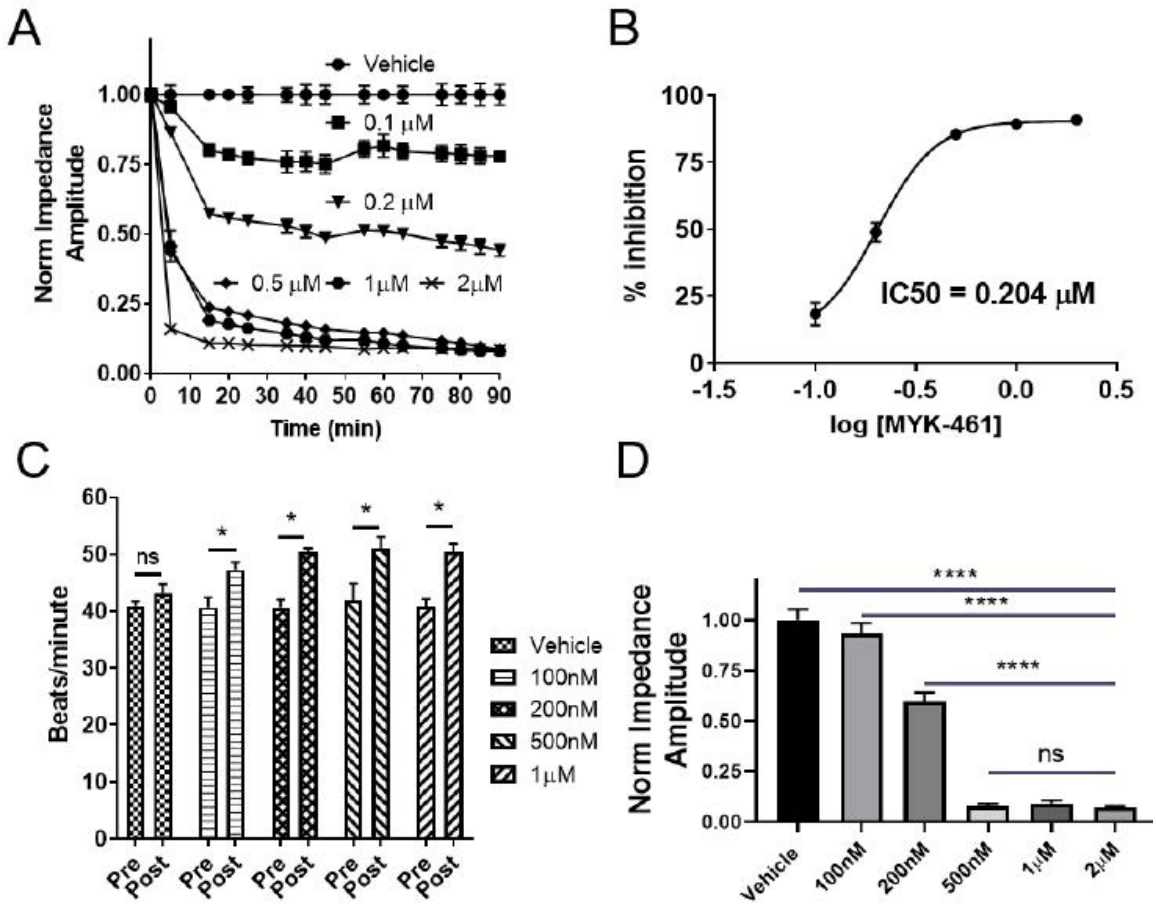


Figure 18. Effect of MYK-461 treatment on contraction of human iPSC-CMS in an impedance assay
A: Dose- and time-dependence of effects of MYK-461. B: Dose-response relationship for MYK-461 (IC50 = 0.204). C: Spontaneous beat rate of iPSC-CMs following treatment with MYK-461. D: MYK-461 effects on impedance amplitude in electrically stimulated (1 Hz) iPSC-CMs. * indicates $p \leq 0.05$, **** indicates $p \leq 0.0001$, ns indicates nonsignificant differences. Panels A and D display impedance values normalized for each sample to pre-drug baseline and post-drug vehicle treatment.

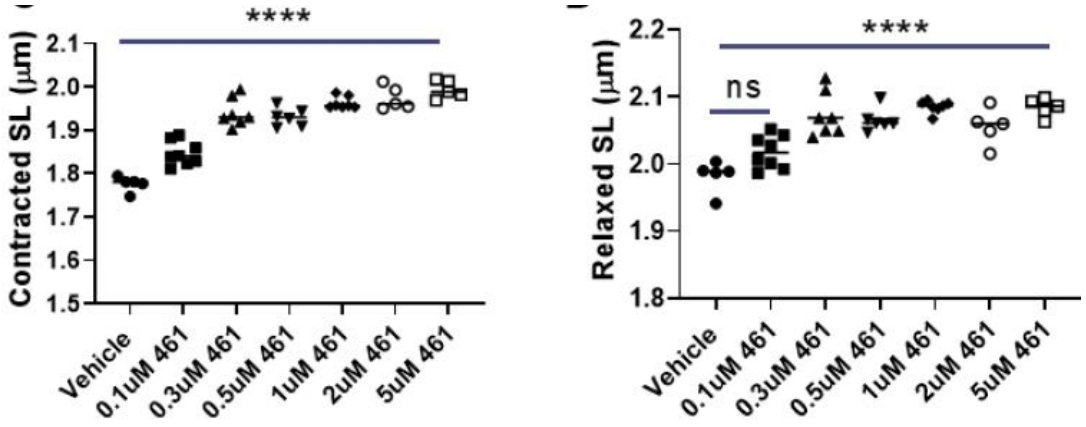


Figure 19. Effect of MYK-461 on sarcomere length during contracted and relaxed sarcomere

4.1.3. *In vivo* studies

4.1.3.1 Pharmacodynamic study of single oral dose of MYK-461 to mice

This non-GLP study (#NC-20-0056) was conducted at MyoKardia, Brisbane, CA (dated 11/27/20020). The study evaluated the cardiac performance of a single oral dose of MYK-461 in conscious healthy mice.

Methods

Healthy male 129s6/SvEv mice (20-30 g, > 8 weeks of age; (b) (4) were assigned into 3 set of experiments with each animal receiving a single oral dose of MYK-461. All dosing was performed at least 24 hours after the baseline assessments to minimize the effects of anesthesia. For M01 and M03: vehicle solution was prepared with dimethylacetamide (DMA), PEG400, and 30% β-cyclodextrin (dissolved in 20 ml of water) in a 5:25:70 ratio. For M02: MYK-461 was homogenized into a 0.5% methylcellulose solution and was administered as a 10 ml/kg suspension.

The first set of animals (M01) received 5, 10, 15, and 20 mg/kg.
The second set of animals (M02) received 1.25, 2.5, 5, 7.5, and 20 mg/kg.
The final group of animals (M03) received 1.25, 2.5, 5, and 7.5 mg/kg.

Under isoflurane anesthesia, cardiac function was evaluated by high-resolution transthoracic echocardiography (TTE) at 2 time points, once prior to dosing (establishing baseline) and at three hours post-dosing (a time when exposures are known to approach steady-state and peak). The measurements included LV internal dimensions (LVID) as well as left-ventricular fractional shortening (FS), an index of systolic performance, and the heart rate. FS was defined as the end-diastole normalized change in internal dimensions/diameter of the left ventricle between end-systole (LVIDs) and end diastole (LVIDd) (i.e., FS = 100· [LVIDd – LVIDs]/LVIDd). From these dimensions, LV volumes were derived assuming a Teichholz model (LVV = 7·[2.4+ LVid]-1·LVid3), and ventricular cardiac-output (CO_{LV}) was estimated from the derived volumes (COLV = [EDV -ESV. In a subset of animals (M01), the diameter of the aorta as well as the velocity time integral (VTI) at the level of the left-ventricular outflow tract (LVOT) (via pulsed-wave Doppler) were used measure to derive forward-flow and cardiac output (CO_{aorta}).

Following measurements, a blood sample was taken by direct cardiac puncture and the animals were humanely euthanized. A sample of left ventricle was collected for the determination of MYK-461 exposures in the heart. Additionally, in a subset of animals (M01), samples of fast- (extensor digitorum longus) and slow-twitch skeletal muscles

(soleus) were collected (flash frozen). Plasma and tissue samples were analyzed to determine the MYK-461 concentrations.

Results

Following oral administration of MYK-461, dose-dependent reductions in fractional shortening (Fig. 20 Right) that were linearly predicted by both circulating (Fig. 20 Left, 21 Left) and LV (Fig. 21 Right) exposures. At 2.5 mg/kg, MYK-461 led to exposures of 595 ± 115 ng/mL, which corresponded with significant reductions in FS (−45% vs. pre-dose). Reductions in FS reached statistical significance at doses ≥ 2.5 mg/kg (Fig. 20 Right). Reduction in FS was associated with increased LV chamber dimensions in both systole and diastole. Reductions in cardiac output were observed at doses greater than 5 mg/kg (Table 8).

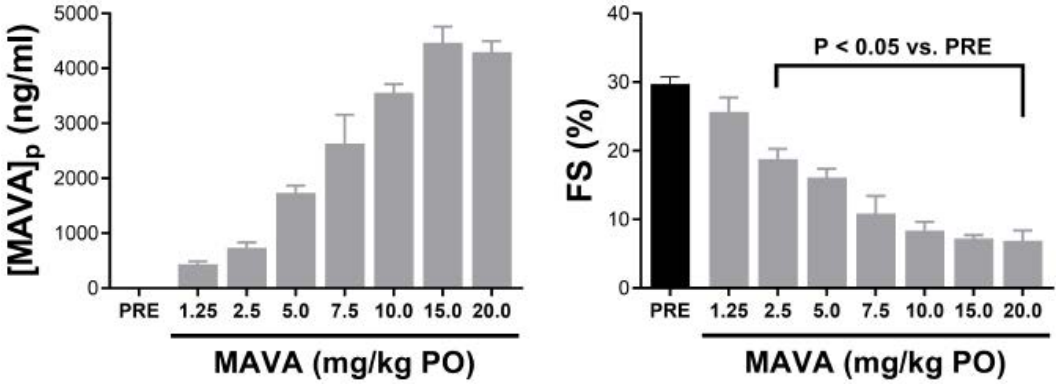


Figure 20. Circulating plasma concentrations (left) and left-ventricular fractional shortening (right) following oral administration of MYK-461 (MAVA) mice

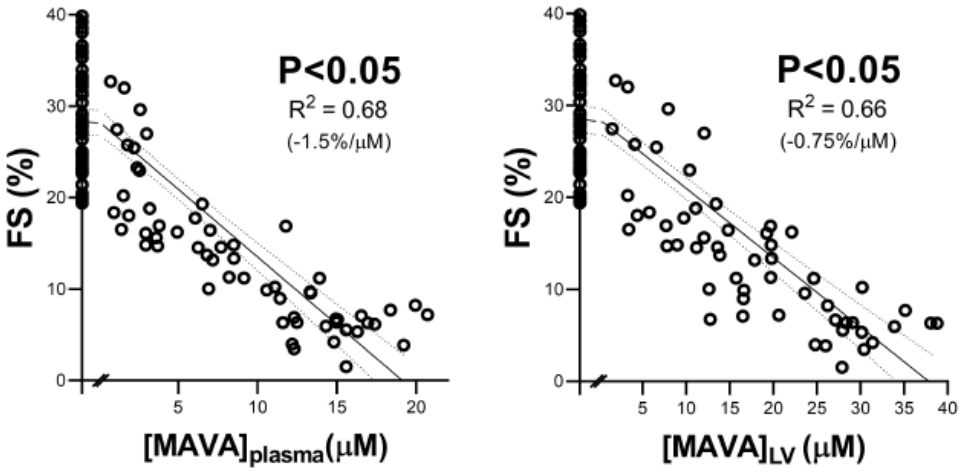


Figure 21. Plasma (left) and left-ventricular (right) concentrations linearly predicted left-ventricular FS following MYK-461 (MAVA) administration to mice

Table 8. Cardiac effects of MYK-461 (mavacamten) (single oral dose) in healthy mice

[MAVACAMTEN]		Echocardiography					
Plasma	LV	HR	LVIDs	LVIDd	FS	CO_{LV}	CO_{aorta}
μM	*μM*	*bpm*	*mm*	*mm*	*%*	*mL/min*	*mL/min*
PRE (n = 48)							
N/A		400.1 ± 0.8	2.6 ± 0.0	3.6 ± 0.0	29.7 ± 0.1	12.4 ± 0.1	23.5 ± 0.2
1.25 mg/kg (n = 7)							
1.6 ± 0.1	3.1 ± 0.2	425.4 ± 9.8	2.9 ± 0.0	3.9 ± 0.0	25.6 ± 0.8	15.7 ± 0.1	N/D
Change vs. PRE (%)		*-1 ± 2*	*20 ± 3#*	*7 ± 2*	*-22 ± 2#*	*-26 ± 12*	-
2.5mg/kg (n = 8)							
2.7 ± 0.1	8.4 ± 0.4	440.6 ± 5.5	3.3 ± 0.0*	4.0 ± 0.0*	18.8 ± 0.5*	12.1 ± 1.0	N/D
Change vs. PRE (%)		*8 ± 1*	*40 ± 4#*	*12 ± 2#*	*-43 ± 2#*	*66 ± 31*	-
5 mg/kg (n = 13)							
6.4 ± 0.1	14.5 ± 0.3	482.2 ± 2.8*	3.2 ± 0.0*	3.8 ± 0.0	16.1 ± 0.4*	10.6 ± 0.2	19.3 ± 1.0
Change vs. PRE (%)		*27 ± 1#*	*27 ± 2#*	*7 ± 1#*	*-42 ± 1#*	*-6 ± 2*	*-5 ± 12*
7.5 mg/kg (n = 8)							
9.6 ± 0.7	21.5 ± 1.2	552.1 ± 6.7*	3.6 ± 0.1*	4.0 ± 0.0*	10.8 ± 0.9*	10.1 ± 0.8	N/D
Change vs. PRE (%)		*14 ± 6#*	*57 ± 4#*	*15 ± 2#*	*-70 ± 2#*	*-36 ± 8*	-
10 mg/kg (n = 9)							
13.0 ± 0.2	22.3 ± 0.7	541.9 ± 5.9*	3.7 ± 0.0*	4.1 ± 0.0*	8.4 ± 0.4*	7.2 ± 0.3*	6.7 ± 0.4*
Change vs. PRE (%)		*29 ± 1#*	*47 ± 3#*	*13 ± 1#*	*-69 ± 2#*	*-41 ± 3#*	*-68 ± 6#*
15 mg/kg (n = 8)							
16.3 ± 0.4	27.1 ± 0.8	530.4 ± 2.9*	3.8 ± 0.0*	4.1 ± 0.0*	7.2 ± 0.2*	6.4 ± 0.2*	8.9 ± 0.4*
Change vs. PRE (%)		*47 ± 2#*	*32 ± 2#*	*7 ± 1*	*-71 ± 1#*	*-42 ± 2#*	*-60 ± 5#*
20 mg/kg (n = 6)							
15.7 ± 0.3	33.8 ± 1.5	526.4 ± 6.1*	3.8 ± 0.0*	4.1 ± 0.0*	6.9 ± 0.5*	6.2 ± 0.5*	6.5 ± 0.7*
Change vs. PRE (%)		*42 ± 2#*	*40 ± 2#*	*11 ± 2#*	*-74 ± 2#*	*-44 ± 5#*	*-78 ± 6#*

Data are mean ± SEM; relative (%) change vs. paired pre-dosing values (i.e., vs. their own baseline) in *italics*.

*: $P < 0.05$ vs overall baseline (PRE); #: $P < 0.05$ vs paired baseline (PRE).

HR: heart rate; LVIDs and LVIDd: left-ventricular internal dimensions at end-systole and end-diastole (respectively); FS: left-ventricular fractional shortening; CO: cardiac output. N/D: not done or collected.

4.1.3.2 Acute and chronic effects of myosin inhibition on mouse models of thin-filament pathogenic troponin mutations

This non-GLP study (#NC-20-0066) was conducted at MyoKardia, Brisbane, CA (dated 12/18/20020). The systolic and diastolic effects of myosin inhibition by MYK-461 was evaluated in mice expressing mutations in the cardiac troponin complex (R92L, R92W and R193H), linking to hypertrophic cardiomyopathy. The pathogenic HCM mutations in troponin complex are presented with a marked diastolic dysfunction with minimal fibrosis.

Methods

Two sets of studies were performed. 1) Acute effects of MYK-581, an analog of MYK-461 in mice expressing cardiac troponin I (cTnI) where arginine (R) at position 193 had been replaced with a histidine (H, R193H), a mutation linked to restrictive cardiomyopathy in humans, and 2) a chronic experiment assessing the effects of MYK-461 given orally for 6 months to mice expressing cardiac troponin T (cTnT) with missense mutations linked to hypertrophic cardiomyopathy, replacing R at position 92 by either leucine (R92L) or tryptophan (R92W).

Study 1: Wild-type and transgenic cTnI R193H female mice ($n \geq 7$/group) were acutely administered MYK-581 (0.3 and 1.0 mg/kg) or vehicle control; systolic and diastolic cardiac function were measured via high-resolution echocardiography 2 h post-dose.

Study 2: WT and transgenic cTnT mice (both R92W and R92L) were either administered MYK-461 (targeting 0.83 mg/kg/day in drinking water) for 6 months (beginning at approximately two months of age) or served as untreated time-controls ($n = 5$ per genotype/group). Systolic and diastolic cardiac function were evaluated via high-resolution echocardiography prior to dosing (i.e., baseline) and monthly during the study period. This report summarizes echocardiographic observations taken at 3 and 6 months after the initiation of treatment.

Results

In comparison to WT, transgenic cTnI R193H mouse showed prolonged isovolumic relaxation (IVRT), reduced early diastolic mitral annular tissue velocity (e'), and elevated filling pressures (as estimated by the ratio of the peak E-wave, or early mitral inflow velocity, to e'; E/e') with preserved fractional shortening (FS), consistent with a primary diastolic defect (Table 9). Acute treatment with MYK-581 (0.3 and 1.0 mg/kg) in transgenic mice with a pathogenic HCM mutation in cardiac Troponin I (R193H), dose

dependently shortened IVRT, increased e', and lowered E/e' suggesting improved ventricular filling and decreased end-diastolic pressures. A modest reduction ($p < 0.05$) in FS was observed at 1 mg/kg (Table 9).

Table 9. Cardiac effects of acute myosin-inhibition with MYK-581 in WT and cTnI R193H transgenic mice

	WT			cTnI R193H		
	Vehicle	0.3 mg/kg	1.0 mg/kg	Vehicle	0.3 mg/kg	1.0 mg/kg
FS (%)	50.43 ± 1.78	50.28 ± 1.72	43.05 ± 0.58*	54.34 ± 1.18	58.97 ± 1.88	47.41 ± 1.64+
E/e'	24.86 ± 1.17	25.16 ± 0.91	24.36 ± 1.39	37.67 ± 1.48*	26.60 ± 1.17+	25.13 ± 0.56+
e' (mm/s)	33.88 ± 0.85	34.66 ± 1.13	35.55 ± 0.82	21.48 ± 1.07*	30.39 ± 1.49+	36.37 ± 1.50+
IVRT (ms)	15.18±0.53	15.02±0.42	15.05±0.28	19.4±0.25*	16.49±0.48+	14.82±0.67+

Data are means ± SEM.*: $p<0.05$ vs. WT vehicle; +: $p<0.05$ vs. cTnI R193H vehicle.

FS: left-ventricular fractional shortening; E/e': ratio of early peak mitral flow velocity to early mitral annular tissue velocity (during diastole); e': early diastolic mitral annular tissue velocity; IVRT: left-ventricular isovolumic relaxation time; WT: wild type. cTnI: cardiac troponin I.

Transgenic cTnT mutant mice relative to WT showed hyperdynamic contraction (increased FS) and impaired relaxation with markedly ($p < 0.05$) elevated E/e' values contributing to diastolic dysfunction (Table 10 and Table 11). In these animals, treatment with MYK-461 induced reductions in left-ventricular filling pressures that were likely mediated by a normalization of early filling patterns, as treatment e' velocities, E-wave deceleration times (DT), and E/A ratios, reflecting early (passive) to late (atrial) mitral inflow patterns. These salutary diastolic effects were observed while preserving FS. Plasma concentrations of MYK-461 ranged between 191 and 610 ng/mL (0.70 to 2.23 µM) (Table 10 and Table 11).

Table 10. Cardiac effects of chronic myosin-inhibition with MYK-461 in cTnT R92W transgenic mice

Time-Point	3-Month			6-Month		
Group	WT	cTnT R92W		WT	cTnT R92W	
		CTRL	MAVA		CTRL	MAVA
FS (%)	26.7 ± 1.4	39.2 ± 1.8*	35.6 ± 1.8*	26.8 ± 1.1	40.0 ± 0.9*	39.4 ± 2.8*
E/e'	-33.5 ± 2.1	-50.4 ± 4.0*	-33.1 ± 2.1#	-33.3 ± 1.2	-45.4 ± 3.0*	-35.2 ± 1.0#
e' (mm/s)	-25.3 ± 1.1	-17.6 ± 1.4*	-22.3 ± 1.3#	-25.8 ± 0.6	-18.4 ± 0.7*	-22.5 ± 1.8
DT(ms)	28.2 ± 0.9	20.3 ± 0.2*	25.4 ± 0.8#	28.7 ± 1.1	21.7 ± 1.2*	26.1 ± 1.3#
E/A	1.3 ± 0.0	2.8 ± 0.6*	1.5 ± 0.2#	1.3 ± 0.0	1.9 ± 0.5	1.6 ± 0.2

Table 11. Cardiac effects of chronic myosin-inhibition with MYK-461 in cTnT R92L transgenic mice

Time-Point	3-Month			6-Month		
Group	WT	cTnT R92L		WT	cTnT R92L	
		CTRL	MAVA		CTRL	MAVA
FS (%)	26.7 ± 1.4	40.3 ± 1.2*	39.9 ± 1.8*	26.8 ± 1.1	43.2 ± 1.9*	40.8 ± 0.8*
E/e'	-33.5 ± 2.1	-42.4 ± 4.1*	-29.1 ± 1.6#	-33.3 ± 1.2	-45.0 ± 2.0*	-36.7 ± 1.3#
e' (mm/s)	-25.3 ± 1.1	-20.0 ± 1.3	-27.2 ± 2.6#	-25.8 ± 0.6	-19.7 ± 1.5*	-20.4 ± 1.0
DT(ms)	28.2 ± 0.9	22.6 ± 0.8*	24.3 ± 1.2	28.7 ± 1.1	22.7 ± 0.8*	26.1 ± 1.4
E/A	1.3 ± 0.0	1.6 ± 0.1	1.5 ± 0.3	1.3 ± 0.0	1.6 ± 0.1	1.2 ± 0.1

Footnotes for Tables 10 and 11
Data are means ± SEM. *: p<0.05 vs. WT; #: p<0.05 vs. CTRL.
FS: left-ventricular fractional shortening; E/e': ratio of early peak mitral flow velocity to early mitral annular tissue velocity (during diastole); e': early diastolic mitral annular tissue velocity; DT: early peak mitral flow velocity (E-wave) deceleration time; E/A: ratio of early (E) and late/atrial (A) peak mitral flow velocities. WT: wild type. CTRL: untreated, and MAVA: receiving MYK-461 at ~0.83 mg/kg/d for 6 months (drinking water; targeted) reaching 1.42 ± 0.07 ng/mL and 1.39 ± 0.23 ng/mL for R92W; and 1.42 ± 0.08 ng/mL and 1.15 ± 0.13 ng/mL for R92L and at 3- and 6-months. cTnT: cardiac troponin T.

4.1.3.3 Pharmacodynamic study of single oral dose of MYK-461 to rats

This non-GLP study (#NC-20-0055) was conducted at MyoKardia, Brisbane, CA (dated 11/24/20020). The study evaluated the cardiac performance, and systemic and left-ventricular hemodynamics of a single oral dose of MYK-461 in conscious and anesthetized (via invasive catheterization) healthy rats.

Methods

Healthy male Sprague Dawley rats were chronically instrumented (telemetered) for the assessment of systemic (arterial) and left-ventricular pressures. A subset of animals assigned to intravenous study were chronically instrumented with an indwelling femoral venous catheter and under isoflurane anesthesia received continuous intravenous infusion of MYK-461 (results of this study are not described except for a single report, see Fig. 22B). Under isoflurane anesthesia, cardiac function/geometry were recorded non-invasively using high-resolution transthoracic echocardiography (TTE) at two separate time-points/days: once prior to dosing (i.e., at baseline) and at 3 hr postdosing (day 0), a time when exposures are known to approach steady-state and peak

responses are expected. A set of conscious rats received MYK-461 orally at 3 dose levels, LOW (1 and 2 mg/kg), MID (4 mg/kg), or HIGH (8 and 10 mg/kg).
Samples of the left-ventricle, fast- (extensor digitorum longus) and slow-twitch skeletal muscles (soleus) were collected (flash frozen) for the determination of mavacamten exposures in the heart and skeletal muscles (results of this study are not given).
Plasma, tissue, and blood samples were analyzed to determine the mavacamten concentration(s).

In another set of conscious telemetered rats, cardiovascular responses to acute myosin modulation were studied in the presence of MYK-461 at one of two dose-levels (1 or 2.0 mg/kg, oral) and its modulation with β-adrenergic receptor blockade by metoprolol succinate (50 mg/kg, oral). In these experiments, systemic/LV hemodynamics were recorded continuously via telemetry in conscious free-roaming animals both prior to (up to 1 hour) and following (up to 22 hours) each dosing (circadian variations).

Results

In healthy conscious rats, MYK-461 dose-dependently (1 to 10 mg/kg) induced myosin inhibition resulting in both significant reductions in systolic contractile indices (reduction in fractional shortening and functional (dP/dt max) depression) and increased ventricular chamber dimensions (end diastolic volume) (Fig. 22).

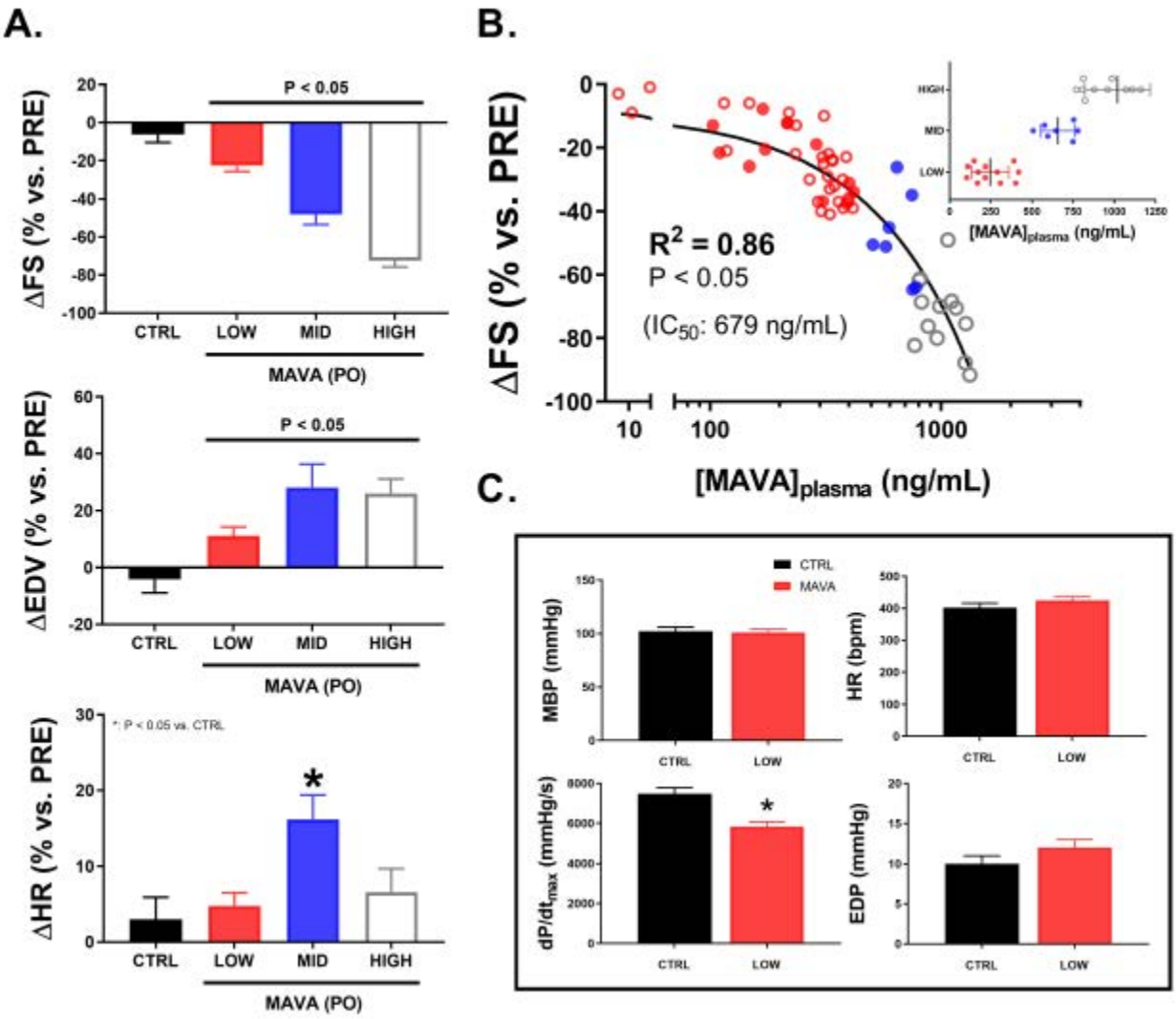


Figure 22. Effect of MYK-461 on cardiac function and hemodynamics following acute administration to healthy rats.
Panel A: Oral administration of MYK-461 (MAVA) dose-dependently decreased fractional shortening (FS, top) and recruited preload (EDV, middle), despite mild/moderate cardio-acceleration (HR, bottom). Panel B: MAVA plasma exposure increased with escalating doses (inset) and predicted the degree of induced functional attenuation. Panel C: Mean systemic (MBP) and LV end diastolic (EDP) pressures were preserved (no changes) following 1 and 2 mg/kg MAVA administration, despite marked functional (e.g., dP/dtmax) depression (and increased EDV, see A). LOW (1 and 2 mg/kg PO, n = 7 and 5, respectively, red with closed circle; 0.8 mg/kg/h IV red with open circles), MID (4 mg/kg PO, n = 7; blue), or HIGH (8 and 10 mg/kg PO, n = 6/each; white). Data are mean ± SEM, showing either absolute values or changes (vs. baseline, PRE) at 3 hours postdosing. dP/dtmax: peak rate of left ventricular pressure development during systole; HR: heart rate; EDV: (estimated) left ventricular end-diastolic volume; MBP: mean systemic (arterial) pressure; EDP: left-ventricular end-diastolic pressure; LV: left ventricular; PRE: predose; CTRL: vehicle. *: P < 0.05 vs PRE or CTRL.

Plasma exposure increased with increasing doses (245 ± 33, 658 ± 40, and 1019 ± 55 ng/mL respectively at low, mid and high doses with an estimated IC50 of 679 ng/mL)

and predicted the degree of induced functional attenuation. At 1 and 2 mg/kg, end-diastolic pressures were preserved following MYK-461 administration, despite marked functional depression and increased EDV (Table 12).

Table 12. Cardiac effects of MYK-461 (single oral dose) in conscious healthy rats

		Echocardiography					
[MAVACAMTEN]		BASELINE (PRE)			DOSE (+3HR)		
Plasma	LV	FS	HR	EDV	FS	HR	EDV
µM	*µM*	*bpm*	*bpm*	*mL*	*bpm*	*bpm*	*mL*
VEHICLE (n = 8)							
N/A		45.7 ± 1.6	369 ± 8	270 ± 13	42.6 ± 1.5	380 ± 13	256 ± 12
Change vs. PRE (%)		-	-	-	*-6 ± 4*	*3 ± 3*	*-4 ± 5*
LOW (1 mg/kg and 2 mg/kg, n = 12)							
0.9 ± 0.1	11.8 ± 1.3	46.5 ± 1.4	383 ± 4	264 ± 8	34.9 ± 2.2	402 ± 9	292 ± 8
Change vs. PRE (%)		-	-	-	*-23 ± 3*$^{*,\#}$	*5 ± 2*$^{\#}$	*11 ± 3*$^{*,\#}$
MID (4 mg/kg, n = 7)							
2.4 ± 0.1	29.6 ± 1.5	43.0 ± 1.7	382 ± 8	264 ± 20	22.6 ± 3.0	443 ± 8	329 ± 8
Change vs. PRE (%)		-	-	-	*-48 ± 5*$^{*,\#}$	*16 ± 3*$^{*,\#}$	*28 ± 8*$^{*,\#}$
HIGH (8 and 10 mg/kg, n = 13)							
3.7 ± 0.2	46.7 ± 3.0	45.1 ± 1.4	378 ± 7	271 ± 8	12.3 ± 1.5	402 ± 10	328 ± 11
Change vs. PRE (%)		-	-	-	*-73 ± 3*$^{*,\#}$	*7 ± 3*	*26 ± 5*$^{*,\#}$

Data are mean ± SEM; relative (%) change vs. paired pre-dosing values (i.e., vs. their own baseline) in *italics*.

*: $P < 0.05$ vs vehicle (CTRL); #: $P < 0.05$ vs paired baseline (PRE). ECHO/PK-PO group.

HR: heart rate; EDV: (estimated) left-ventricular internal volume at end-diastole; FS: left-ventricular fractional shortening. N/A: not applicable.

Similar observations were noted in the 2nd set of experiments (conscious telemetered rats). At the 3-hours post-dosing, MYK-461 (at 1 to 2 mg/kg, oral) dose-dependently decreased dP/dtmax, increased heart rate (Fig. 23A) (-19 ± 3%, $P < 0.05$) while preserving EDP and mean systemic blood pressure (Fig. 23 B) (Table 13) (+2 ± 1 mmHg, N.S.). Beta adrenergic blockade with metoprolol decreased heart rate and systemic pressures (Fig. 23B).

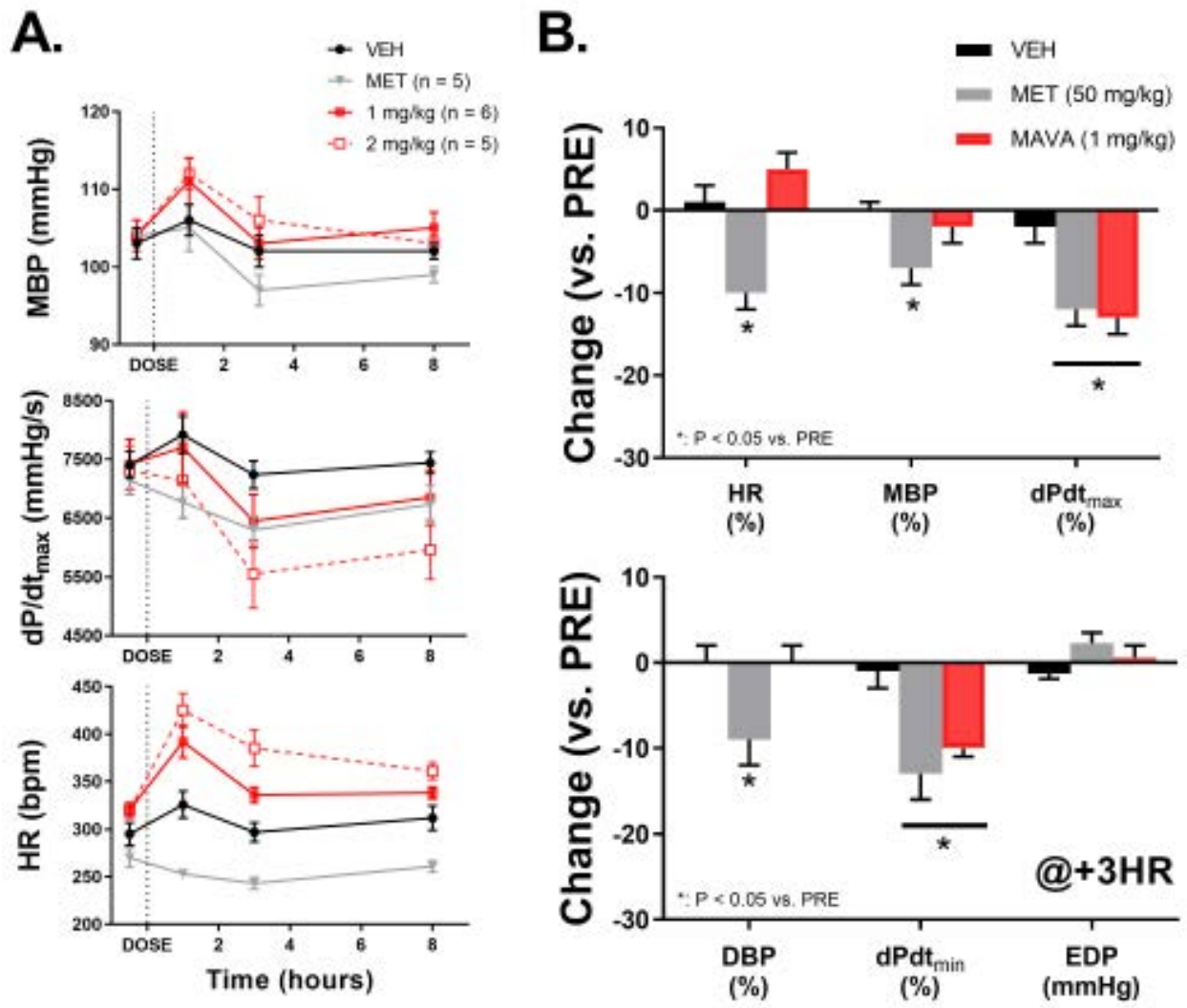


Figure 23. Hemodynamic effects of MYK-461 in conscious telemetered healthy rats.

Panel A: In healthy conscious telemetered rats, oral low-dose MYK-461 (MAVA) administration preserved mean systemic pressures (MBP, top) and dose-dependently decreased the peak rate of LV pressure development (dP/dtmax, middle) with mild/moderate cardio-acceleration (HR, bottom). Panel B: Comparative effects (at +3 hours postdosing) of MAVA and metoprolol (MET) on heart rate (HR), mean (MBP) and diastolic systemic pressures (DBP), as well as in LV end-diastolic pressures (EDP) and peak-rates of pressure change (dP/dtmax and dP/dtmin); at matched reductions in dP/dtmax, MAVA preserved systemic hemodynamics. Data are mean ± SEM, showing either absolute values both predose and postdosing (DOSE) or changes (vs PRE) at 3 hours postdosing. dP/dtmax and dP/dtmin: peak rates of left ventricular pressure increase or decrease during systole and diastole (respectively), LV: left ventricular; PRE: predose; VEH: vehicle. *: P < 0.05 vs PRE.

Table 13. Acute hemodynamic effects of MYK-461 in conscious telemetered healthy rats

Group	TIME	MBP	DBP	PP	HR	LV-EDP	LV-dPdt$_{max}$	LV-dPdt$_{min}$
		mmHg	*mmHg*	*mmHg*	*bpm*	*mmHg*	*mmHg/s*	*mmHg/s*
VEH (n = 11)	PRE	103 ± 2	85 ± 2	40 ± 1	295 ± 12	5 ± 1	7,405 ± 228	-5,725 ± 131
	+1HR	106 ± 2	89 ± 2	39 ± 1	326 ± 14	5 ± 1	7,918 ± 313	-6,270 ± 223
	+3HR	102 ± 2	85 ± 2	39 ± 1	297 ± 10	3 ± 1	7,237 ± 239	-5,673 ± 177
	+8HR	102 ± 1	84 ± 1	39 ± 1	312 ± 13	5 ± 1	7,443 ± 186	-5,787 ± 162
	Δ@+3HR	*0 ± 1%*	*0 ± 2%*	*-2 ± 2%*	*1 ± 2%*	*-1 ± 1*	*-2 ± 2%*	*-1 ± 2%*
MAVA (n = 11)	PRE	104 ± 1	87 ± 1	39 ± 1	320 ± 5	5 ± 3	7,370 ± 285	-5,653 ± 208
	+1HR	111 ± 2	96 ± 2	33 ± 1	407 ± 13	9 ± 3	7,448 ± 428	-5,978 ± 324
	+3HR	104 ± 2	90 ± 2	32 ± 2	358 ± 12	7 ± 3	6,041 ± 371	-4,933 ± 289
	+8HR	104 ± 1	89 ± 1	34 ± 1	348 ± 6	6 ± 2	6,447 ± 355	-5,420 ± 260
	Δ@+3HR	*0 ± 1%*	*3 ± 2%*	*-17 ± 4%**	*12 ± 3%**	*2 ± 1*	*-19 ± 3%**	*-13 ± 3%**
METO (n = 6)	PRE	104 ± 1	86 ± 2	42 ± 2	270 ± 10	7 ± 2	7,137 ± 234	-5,581 ± 62
	+1HR	105 ± 3	87 ± 2	41 ± 2	253 ± 4	8 ± 1	6,767 ± 275	-5,399 ± 250
	+3HR	97 ± 2	78 ± 2	43 ± 2	243 ± 6	9 ± 2	6,298 ± 231	-4,859 ± 142
	+8HR	99 ± 1	82 ± 1	40 ± 2	261 ± 6	9 ± 2	6,728 ± 328	-5,285 ± 203
	Δ@+3HR	*-7 ± 2%**	*-9 ± 2%**	*4 ± 5%*	*-10 ± 2%**	*2 ± 1*	*-12 ± 2%**	*-13 ± 3%**

Data are mean ± SEM both before (PRE) and post-dosing (at +1, +3, and +8-hours); relative (%) change vs. paired pre-dosing values in *italics*. *: $P < 0.05$ vs vehicle at the +3HR time-point. MAVA: mavacamten at 1.0 (n = 6) or 2.0 mg/kg PO (n = 5). METO: metoprolol at 50 mg/kg PO (n = 6)

MBP, DBP, and PP: arterial (systemic) mean, diastolic, and calculated pulse pressure (respectively); HR: heart rate; EDP: left-ventricular end-diastolic pressure; **dP/dt$_{max}$ and dP/dt$_{min}$**: peak rates of left ventricular pressure increase/decrease during systole and diastole (respectively).

Additionally, the effect of MYK-461 on circadian (24 hr duration)-induced autonomic activation was studied in the absence and presence of β-adrenergic receptor blockade in telemetered rats. The onset of the dark photo-period (night) triggered marked increases in heart rate, as well as in the peak rates of left-ventricular pressure development (dP/dtmax) and decay (dP/dtmin), consistent with circadian autonomic activation and functional recruitment (Fig. 24). MYK-461 administration preserved the intrinsic chronotropy-dependent circadian (light-cycle induced) recruitment of systolic function while facilitating diastolic recruitment (i.e., dP/dtmax and dP/dtmin increased with heart rate). On the other hand, β-adrenergic blockade with metoprolol blunted functional chronotropic recruitment. As a result, the onset of the dark photo-period post-dosing triggered negligible EDP changes in vehicle- (+1 ± 1 mm Hg) and MYK-461-treated rats (-1 ± 1 mm Hg, not significant), thus led to detectable elevations (+4 ± 1 mm Hg, $P < 0.05$) in β-AR blocked animals (Fig. 24). According to the sponsor, these observations could have important implications for the recruitability of cardiac function during exercise in HCM.

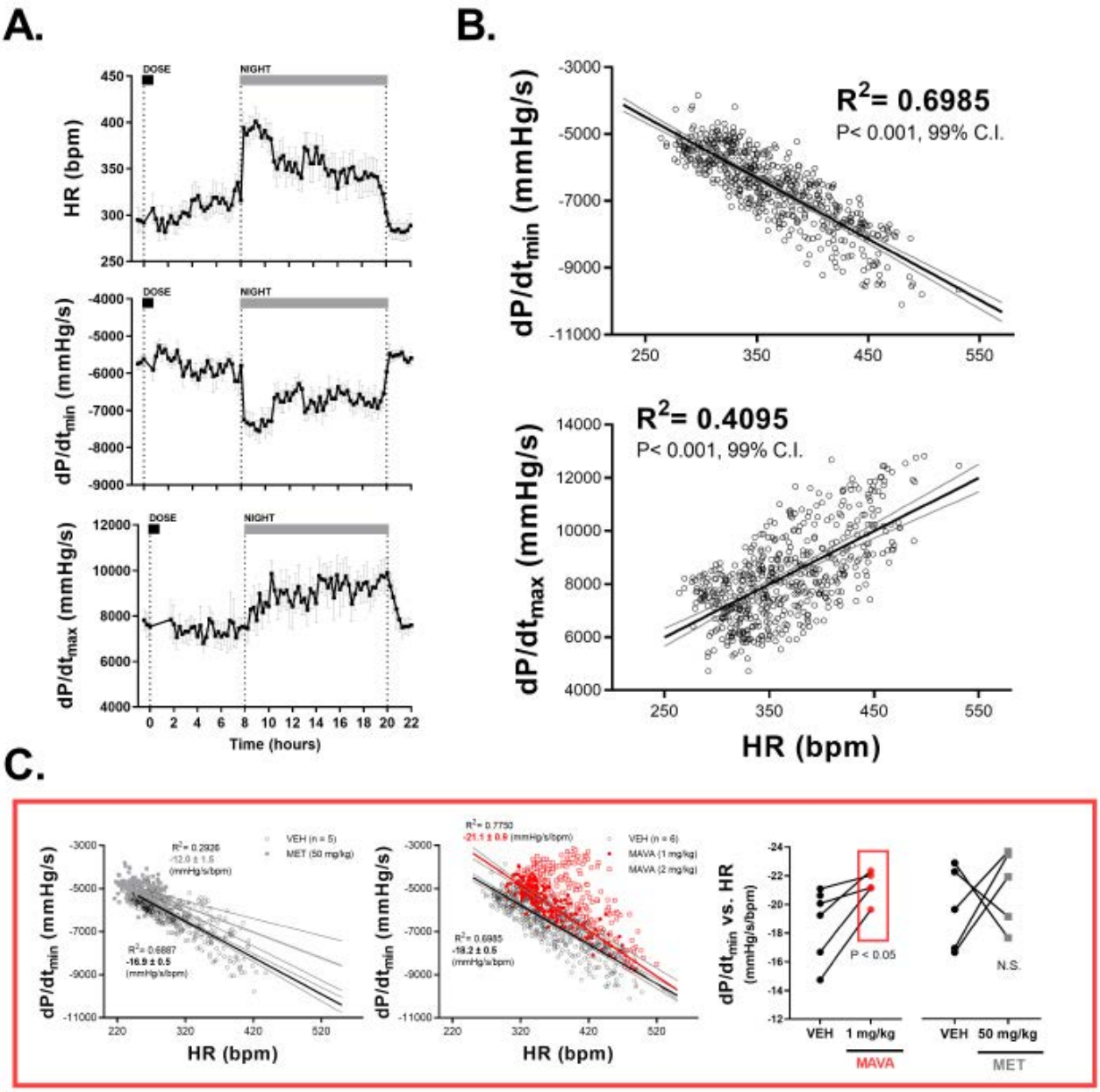


Figure 24. Hemodynamic effects of MYK-461 in conscious telemetered healthy rats.

Panel A: In healthy conscious telemetered rats, the onset of the dark photo-period (night) triggered marked increased in heart rate (HR, top), as well as in the peak rates of left ventricular pressure development (dP/dtmax, middle) and decay (dP/dtmin, bottom), consistent with circadian autonomic activation and functional recruitment. Panel B: Changes in dP/dtmax (systolic function) and dP/dtmin (diastolic function) were predicted by chronotropic changes. Panel C: MYK-461 (MAVA) treatment (1 to 2 mg/kg PO), preserved the intrinsic circadian heart rate changes, but facilitated diastolic recruitment, as indicated by steeper slopes of the dP/dtmin vs. HR relationships. In contrast, metoprolol (50 mg/kg PO) blunted chronotropic and functional recruitment.

4.1.3.4 Effects of dobutamine and levosimendan on cardiac performance on depressed ventricular performance induced by MYK-461 in rats

This non-GLP study (#NC-20-0057) was conducted at MyoKardia, Brisbane, CA (dated 12/18/2020). The study evaluated the cardiac performance as evaluated by high-resolution transthoracic echocardiography (TTE) of a single oral dose of MYK-461 in conscious healthy rats. Additionally, the study evaluated the ability of two inotropes, dobutamine and levosimendan (a phosphodiesterase-3 inhibitor) in counteracting MYK-461-mediated inhibition of LV contractility in rats.

Methods

Healthy male Sprague Dawley rats (300-400 g) were chronically instrumented with an indwelling femoral venous catheter for receiving intravenous doses of dobutamine and levosimendan. Under isoflurane anesthesia, cardiac function/geometry were recorded non-invasively using high-resolution transthoracic echocardiography (TTE) predosing (baseline), three hours following a single oral dose of MYK-461 (4 mg/kg in 0.5% methylcellulose solution), and before and at steady-state during the inotropic challenge. Dobutamine (10 ug/kg/min IV for 10 min) or levosimendan (0.3 µmol/kg IV over 20 min) challenges were given approximately 3 hours following the administration of MYK-461. Blood samples were collected for the purpose of circulating exposure determination. In addition, samples of the left-ventricle as well as of fast- (extensor digitorum longus) and slow-twitch skeletal muscles (soleus) were collected (flash frozen) for the determination of MYK-461 exposures in the heart and skeletal muscles (results not given).

Results

In healthy conscious rats, single oral dose of MYK-461 (4 mg/kg) produced significant and consistent reductions in fractional shortening (-54% vs. pre-dosing, $P < 0.05$) and ventricular stroke volume (SV, -40% vs. pre-dosing, $P < 0.05$). These effects were observed at circulating plasma concentration of 417 ± 77 ng/mL. The MYK-461-induced reductions in systolic cardiac performance and forward flow were accompanied by marked increases in both end-systolic and end-diastolic dimensions/volumes with a moderate ($P > 0.05$) increase in heart rate (Table 14).

Intravenous infusion of both dobutamine (Table 15) and levosimendan (Table 16) restored cardiac function indices of systolic function depressed (>50% reduction in ejection fraction) by MYK-461 (Fig. 25).

Table 14. Left-ventricular responses to MYK-461 (Mava, 4 mg/kg PO) in healthy rats

	HR	LVIDs	LVIDd	ESV	EDV	SV	EF	FS	CO
	BPM	Mm	mm	µL	µL	µL	%	%	mL/min
PRE	363 ± 9	4.2 ± 0.2	7.3 ± 0.2	79 ± 7	284 ± 17	208 ± 11	73 ± 1	43 ± 1	75 ± 4
MAVA	386 ± 9	6.2 ± 0.3*	7.7 ± 0.2*	200 ± 18*	316 ± 14*	113 ± 9*	37 ± 4*	19 ± 2*	44 ± 4*
% vs. PRE	*7 ± 3*	*49 ± 5*	*6 ± 1*	*157 ± 19*	*14 ± 3*	*-40 ± 5*	*-46 ± 4*	*-54 ± 4*	*-36 ± 5*

Data are mean ± SEM; relative (%) change vs. paired pre-dosing values in *italics*. *: P < 0.05 vs PRE. MAVA: Approximately 3-hours after mavacamten administration at 4 mg/kg PO (oral gavage), resulting in plasma concentrations of 417 ± 77 ng/mL.

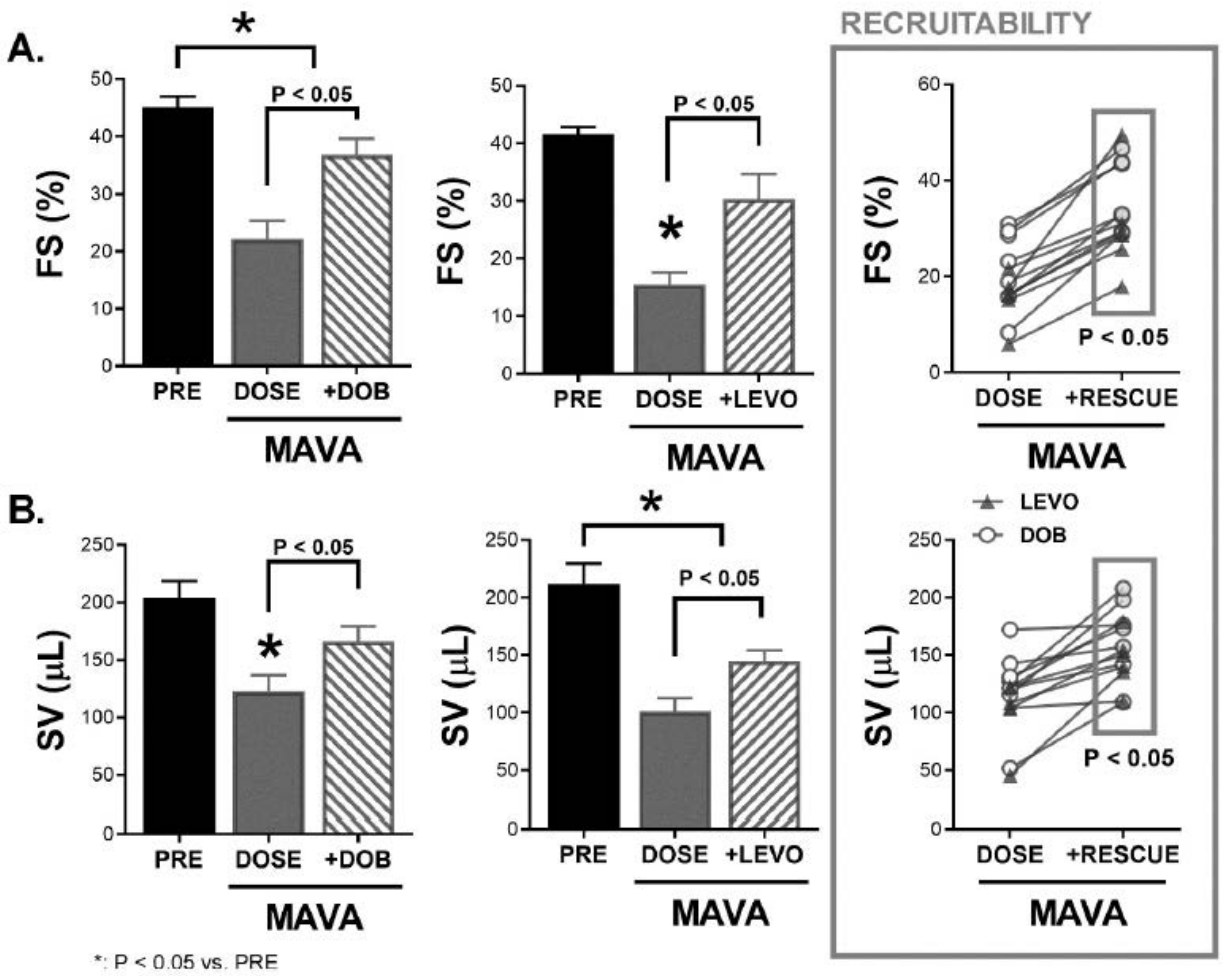
Table 15. Acute effects of dobutamine (DOB) on the MYK-461-induced functional cardiac depression in healthy rats

	HR	LVIDs	LVIDd	ESV	EDV	SV	EF	FS	CO
	BPM	Mm	mm	µL	µL	µL	%	%	mL/min
PRE	378 ± 12	4.0 ± 0.2	7.1 ± 0.3	71 ± 10	269 ± 22	204 ± 15	75 ± 2	45 ± 2	76 ± 3
DOSE	383 ± 13	5.8 ± 0.4	7.5 ± 0.2	173 ± 24*	301 ± 19*	123 ± 14*	43 ± 5	22 ± 3*	47 ± 5*
% vs. PRE	*2 ± 4*	*48 ± 7*	*6 ± 2*	*155 ± 30*	*13 ± 4*	*-38 ± 8*	*-43 ± 7*	*-51 ± 6*	*-38 ± 7*
+DOB	433 ± 22*	4.5 ± 0.3	7.0 ± 0.3	94 ± 14*	260 ± 22	176 ± 15	65 ± 4	37 ± 3*	71 ± 6
% vs. PRE	*15 ± 6*	*12 ± 4*	*-1 ± 3*	*33 ± 11*	*-2 ± 7*	*-13 ± 6*	*-14 ± 3*	*-19 ± 4*	*-6 ± 5*
% vs. DOSE	*13 ± 5#*	*-23 ± 4#*	*-7 ± 3#*	*-44 ± 7#*	*-14 ± 5#*	*50 ± 13#*	*22 ± 3#*	*86 ± 28#*	*24 ± 7#*

Table 16. Acute effects of levosimendan (LEVO) on the MYK-461-induced functional cardiac depression in rats

	HR	LVIDs	LVIDd	ESV	EDV	SV	EF	FS	CO
	BPM	Mm	mm	uL	uL	uL	%	%	mL/min
PRE	346 ± 12	4.4 ± 0.2	7.5 ± 0.3	89 ± 11	301 ± 26	212 ± 17	71 ± 2	42 ± 1	74 ± 8
DOSE	390 ± 12	6.6 ± 0.3	7.8 ± 0.2	231 ± 24*	332 ± 21	101 ± 12*	31 ± 4	15 ± 2*	39 ± 4
% vs. PRE	*13 ± 4*	*52 ± 5*	*5 ± 3*	*166 ± 20*	*12 ± 6*	*-50 ± 7*	*-56 ± 6*	*-63 ± 5*	*-42 ± 9*
+LEVO	375 ± 14	5.0 ± 0.5	7.2 ± 0.3	129 ± 28	274 ± 27	145 ± 9*	55 ± 6	30 ± 4	54 ± 5
% vs. PRE	*9 ± 3*	*16 ± 10*	*-4 ± 3*	*48 ± 26*	*-8 ± 7*	*-30 ± 5*	*-22 ± 8*	*-27 ± 10*	*-24 ± 7*
% vs. DOSE	*-3 ± 4*	*-24 ± 5#*	*-9 ± 3#*	*-46 ± 8#*	*-18 ± 6#*	*58 ± 29#*	*24 ± 4#*	*109 ± 27#*	*15 ± 6#*
+LEVO/F	375 ± 8	5.2 ± 0.4	7.7 ± 0.3	133 ± 23*	319 ± 25	186 ± 14	59 ± 4	33 ± 3	69 ± 5
% vs. PRE	*9 ± 3*	*18 ± 6*	*3 ± 3*	*51 ± 18*	*7 ± 6*	*-10 ± 8*	*-16 ± 6*	*-21 ± 7*	*-2 ± 10*

Data are mean ± SEM; relative (%) change vs. paired pre-dosing values in *italics*. *: P < 0.05 vs PRE (LVID, and EF not studied); #: P < 0.05 vs DOSE (t-test). DOSE: Approximately 3-hours after mavacamten administration at 4 mg/kg PO (oral gavage), resulting in plasma concentrations of 322 ± 38 ng/mL. +LEVO: Acute levosimendan challenge in the setting of mavacamten 0.3 µmol/kg IV over 20min); +LEVO/F: acute volume expansion under +LEVO (0.9% NaCl at 30 mL/kg/hr IV).
HR: heart rate; LVIDs and LVIDd: left-ventricular internal dimensions at end-systole and end-diastole (respectively); ESV and EDV: derived (calculated) left-ventricular volumes at end-systole and end-diastole (respectively); SV: calculated left-ventricular stroke volume; FS: left-ventricular fractional shortening; CO: calculated cardiac output.



IV: intravenous; PO: per os, oral.
In healthy rats, administration of mavacamten (MAVA at 4 mg/kg PO) lead to marked reductions in both fractional shortening (FS, *Panel A*) and stroke volume (SV, *Panel B*) . which were rescued/recruited by acute administration of either dobutamine (+DOB, 10 µg/kg/min IV for 10 min) or levosimendan (+LEVO, 0.3 µmol/kg IV for 20 min) in all mavacamten-treated animals (+RESCUE); LEVO-mediated recruitment was independent of its preload effects. Data are mean ± SEM, showing absolute values predosing/baseline (PRE) and 3 hours (DOSE) following mavacamten administration, as well as during functional rescue (+RESCUE).

Figure 25. Acute effects of dobutamine (DOB) or levosimendan (LEVO) on the MYK-461-induced functional cardiac depression of healthy rats

4.1.3.5 Acute and chronic effects of MYK-461 on cardiac performance and hemodynamics in conscious dogs

This non-GLP study (#NC-20-0058) was conducted at MyoKardia, Brisbane, CA (dated 11/24/20020). The study evaluated the effects of MYK-461 on cardiac performance as well as systemic and left-ventricular hemodynamics following single intravenous, oral or repeated low dose oral administration for 31 days in conscious chronically instrumented healthy dogs. The secondary objective was to determine the relationship of plasma concentrations of MYK-461 to cardiac performance.

Methods

Mongrel dogs (20 months old, weighing 25 to 29 kg) were chronically instrumented (telemetered) for the assessment of systemic (arterial) and left ventricular pressures, coronary blood flow and LV diameter. Also, catheters were placed in the descending thoracic aorta for arterial blood sampling and for blood pressure measurements and in the coronary sinus for myocardial venous blood sampling. Echocardiography was performed for EF, stroke volume, cardiac output and FS measurements. Two independent studies (from two different laboratories) were performed 10 days after the recovery.

1. a) Acute intravenous infusion of 0.3 mg/kg, 0.4 mg/kg, 1.2 mg/kg, or 3.6 mg/kg for 90 min. b) Chronic oral dosing of 45 µg/kg/day for 31 consecutive days.

2. Single oral dose of 1 mg/kg

For IV infusion, MYK-461 was first thoroughly dissolved in DMA, then diluted in saline/PEG400 and filtered through a 0.22 µm filter prior to infusion. For oral study, powder in capsule was swallowed by animals.

Results

Acute intravenous study: The effects of MYK-461 IV infusion on LV systolic function occurred at 10 min after initiation and, peaked at the end of infusion and gradually recovered but it was still depressed 3 hours post infusion. The 1.2 mg/kg IV dose level (Fig. 26) decreased ejection fraction (EF), fractional shortening (FS), and LV+dP/dt. No statistically significant changes in LV Tau and the time to half-maximal LV relaxation (LVRT50) were observed. There were no remarkable changes in LV –dP/dt and coronary blood flow. At the high IV dose (3.6 mg/kg) there was a greater reduction in LV systolic function (-53 % compared to baseline for EF; 22.1% compared to baseline for FS) resulting in fall of arterial pressure (MAP) from 97 mm Hg to 80 mm Hg and systolic

blood pressure (SBP) from 124 mm Hg to 90 mm Hg and an increase in heart rate (80 bpm to 220 bpm) with evidence of agitation in dogs. The changes were most significant after 30 min of infusion (infusion stopped when HR reached 220 bpm at 30 min) and started to recover right after the infusion was stopped but was still significant high 1 h post infusion and returned to normal 3 h post infusion. MYK-461 plasma concentration sampled at the end of infusion and 1 and 3 h post dose showed that it correlated with reduction in LVEF and LVFS. Plasma concentration declined to approximately 50% and 25% of the peak at 1 and 3 h post doses, respectively (Fig. 27).

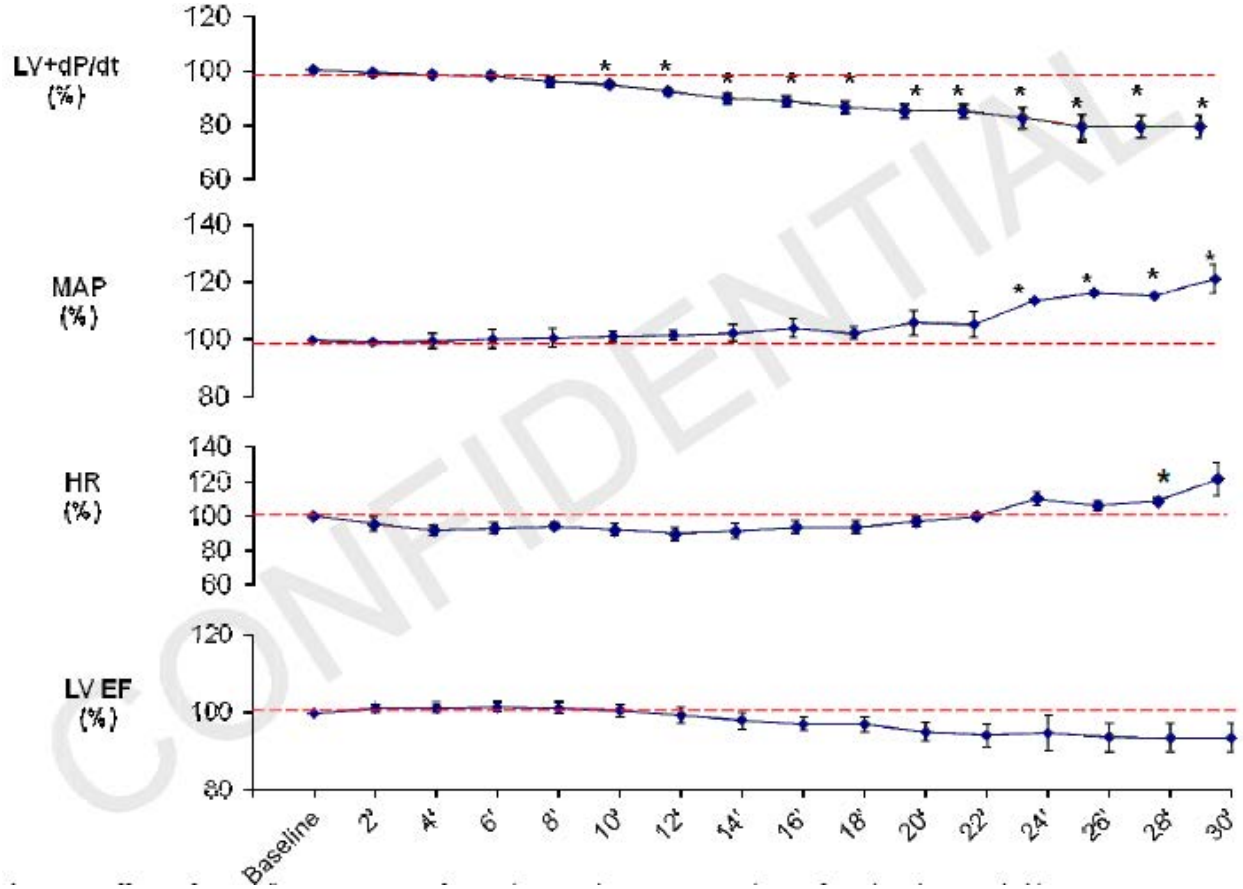


Figure 26. Intravenous effects of MYK-461 on cardiac performance

Effects of 1.2 mg/kg MYK-461 IV infusion dose on the percentage change from baseline LV+dP/dt, MAP, HR and EF at every 2 minutes for the first 30 min in conscious chronically instrumented dogs (n=4).

Chronic oral dose: The results from chronic low oral dose (45 µg/kg/day for 31 days) were more gradual in onset and lesser in magnitude than the IV dose. The effect started 4 days after treatment and peaked and stabilized after 25 days. The plasma concentrations of MYK-461 steadily increased over time, reaching steady-state on day 19 (Fig. 28). Cardiac performance measured as inhibitory effects on LVEF and LVFS

reached maximal effect (85 ± 3.6% of baseline) on day 19 and remained thereof until the end of dosing on day 31 (Fig. 28). There were no significant changes in LV +dP/dt and LV –dP/dt, LV Tau, LV RT50, MAP or heart rate for the duration of the study. At the end of the oral dosing, the hearts were analyzed histologically, and no myocyte hypertrophy was observed. The chronic low oral dose produced an 11% decrease in EF, a 9% decrease in FS and a 17% decrease in LV+dP/dt. This was achieved by limiting the contractility of LV muscle, without significantly affecting diastolic function (Table 17).

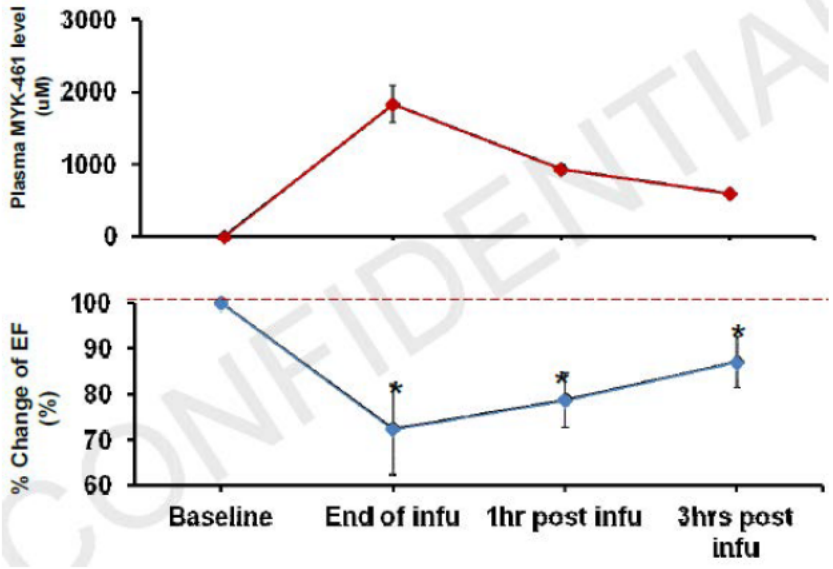


Figure 27. Intravenous effects of MYK-461 on LV ejection fraction
Effects of 1.2 mg/kg MYK-461 IV infusion dose on the average percentage change in LV ejection fraction (n=4) as compared to baseline (bottom) and blood level (n=3) (top) at baseline, end of infusion, 1 hour and 3 hours post infusion in conscious chronically instrumented dogs. The maximum decrease in LV ejection fraction occurred at the end of infusion, it gradually recovered but it was still depressed 3 hours post infusion.

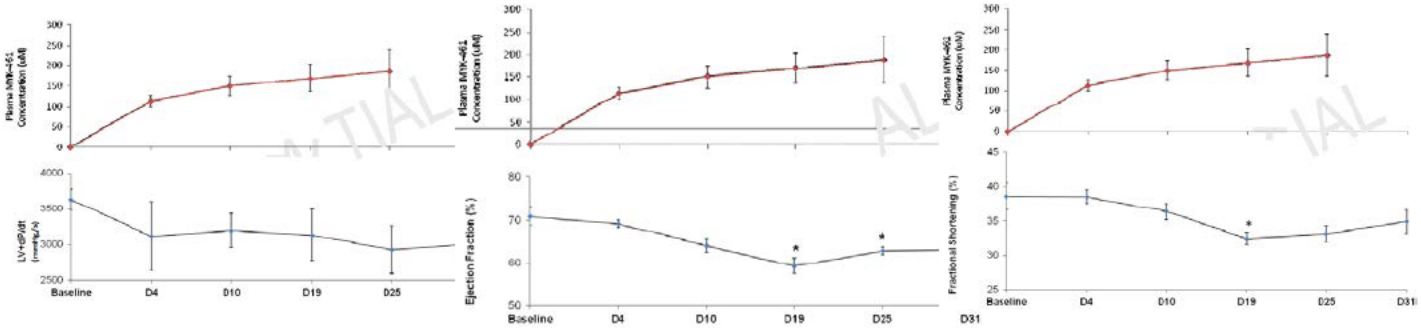


Figure 28. Chronic oral dose effects of MYK-461 on cardiac performance
Effects of 45 µg/kg/day MYK-461 oral dosing for 31 days on LV +dP/dt (left), ejection fraction (center), LV fractional shortening (right) and drug blood levels at baseline (top panel, red color line), at days 4, 10, 19, 25 and 31 in conscious chronically instrumented dogs (n=5). The decrease in cardiac function parameters started on day 4 and continued to decrease until day 19 and it remained depressed until the end of the experiment on day 31. *: denotes statistical significance from baseline.

Table 17. Acute and chronic cardiovascular effects of MYK-461 in conscious healthy dogs

Parameter	ACUTE	CHRONIC
	1.2 mg/kg IV (+1 HR post-dose)	45 µg/kg/day PO (@31 days)
EF (%)	-20 ± 2%↓	-11 ± 0%↓
CO (%)	-6 ± 6%	-24 ± 4%↓
dP/dt_{max} (%)	-25 ± 2%↓	-17 ± 3%↓
HR (%)	+25 ± 8%↑	-10 ± 5%
MAP (%)	+15 ± 6%*	-2 ± 3

EF: left ventricular ejection fraction; CO: cardiac output; dP/dt_{max}: peak rate of left ventricular pressure increase during systole; HR: heart rate; MAP: mean arterial blood pressure; IV: intravenous; PO: oral.
↑, ↓: $P < 0.05$ vs pre-dosing values.
Data are mean ± SEM, showing relative (%) changes from pre-dosing/ baseline values at either 1 hour post-dosing (IV) or at 31 days of dosing (PO).

A single oral dose of 1 mg/kg produced marked effects on LVSP (mm Hg), MAP (mm Hg), PP (mm Hg), dP/dt max (mm Hg/sec), and dP/dtmin (mm Hg/sec) relative to the vehicle group at several time points starting from 2 h to 22 h post dose. Section 4.1.3.6 describes in detail the effect of single oral dose of MVA (1.5 mg/kg) on cardiac indices.

In conclusion, neither acute (IV) nor chronic (PO) administration appeared to affect indices of diastolic function; for instance, in all cases (both studies including acute oral dosing) the time-constant of left-ventricular pressure decay (reflecting ventricular relaxation) remained unchanged postdosing.

4.1.3.6 Cardiovascular effects of a single oral dose of MYK-461 in dogs

This non-GLP study (#NC-20-0059) was conducted at (b) (4) (dated 12/17/20020). The study evaluated the cardiac performance, and systemic and left-ventricular hemodynamics of a single oral dose of MYK-461 in conscious chronically instrumented healthy dogs.

Methods

The studies were performed on Beagle dogs (20 months old, weighing 25 to 29 kg). They were chronically instrumented (telemetered) for providing a single lead ECG and systemic (arterial) and left ventricular pressures. Additionally, a pair of sono-micrometry crystals were secured across the mid-papillary short-axis (endocardium) for the monitoring of antero-posterior internal dimensions (LVid), and derived ventricular volumes. A pneumatic occlude was secured around the inferior/caudal vena cava (IVC) for the generation of LV pressure-volume curves during heterometric auto-regulation (brief IVC occlusion). Two types of studies were performed 14 days after the recovery.

1. A single oral dose of 1.5 mg/kg MYK-461 suspended in 0.5% methylcellulose was administered by gavage. Cardiac function, geometry and systemic/ventricular hemodynamics were examined both before dosing (at baseline) as well as at 1, 3, 8, and 24 h following the onset of treatment. Responses at the 3-h post-dose time-point were compared against those obtained (time-matched) following a single-dose metoprolol tartrate administration (2 mg/kg oral). For reference, time-matched cardiovascular data was also collected in a subset of untreated animals serving as controls.

2. The effects of MYK-461 (1.5 mg/kg) on cardiac reserve were evaluated in the presence of acute β-AR challenges (dobutamine: 2, 5, and 10 µg/kg/min IV). These challenges were performed before/after dosing (+3 h) in control- and MYK-461-treated animals both under normal cardiac physiological conditions and under (mild) concomitant cardio-depression induced via either selective β-AR blockade (metoprolol 0.5 ± 0.1 mg/kg oral tid) or L-type Ca^{2+}-channel blockade (verapamil at 5 ± 1 mg/kg oral tid); pharmacological blockades were established for 7 days prior to the MYK-461 treatment. Both peak and dose-responses were evaluated at steady state.

Plasma concentration of MYK-461 was determined from blood samples collected (via either the jugular and/or a peripheral vein) prior to dosing and at 0.25, 0.5, 1, 3, 8, and 24 h post dose.

Results

After single oral dose of 1.5 mg/kg, mean MYK-461 plasma concentrations measured at 3 and 24 hr post dose were 254 ± 33 ng/mL and 147 ± 30 ng/mL, respectively.

A single oral dose of MYK-461 (1.5 mg/kg) produced marked reductions in systolic contractile indices and increased ventricular chamber dimensions. MYK-461 decreased LV EF (-35%,) and dP/dtmax (-27%), while increasing end-systolic volumes (ESV: +80 ± 7). The reduction in indices of myocardial performance were the result of blunted clinotropy (velocity of contraction occurring pre-ejection) and inotropy (overall power). MYK-461 effectively slowed the velocity of contraction, prolonged the left-ventricular contraction time (pre-ejection period, CT: +25%), but also decreased end-systolic elastance (Ees, -31%), preload-recruitable stroke work (PRSW, - 33%), and myocardial stroke volume (SV: -25%), indicating a reduction in overall myocardial power (all $P < 0.05$ vs. baseline). The functional inhibition of MYK-461 was exposure-dependent, since systolic function was inhibited to a lesser degree at +24-hours than was at +3-hours after dosing (e.g., EF: -13 ± 2 vs. -28 ± 5% and dP/dtmax: -10 ± 3% vs. -28 ± 3%) (Table 18 Fig. 29).

Table 18. Acute hemodynamic and cardiac effects of MYK-461 in conscious dogs at 3 hr post dose (average of both studies)

	EF (%)	dP/dt_{max} (mmHg/s)	CT (ms)	Ees (mmHg/mL)	PRSW (*mmHg)	SV (mL)	MBP (mmHg)	HR (bpm)
PRE	61.2 ± 1.9	3,295 ± 184	37 ± 2	8.7 ± 0.6	93 ± 4	17.6 ± 0.6	108 ± 4	99 ± 3
MAVA	40.1 ± 3.0	2,443 ± 180	41 ± 2	6.1 ± 0.5	63 ± 4	13.4 ± 1.1	110 ± 3	122 ± 4
Change	*-35 ± 4↓*	*-27 ± 2↓*	*25 ± 3↑*	*-31 ± 3↓*	*-33 ± 2↓*	*-25 ± 5↓*	*3 ± 2*	*25 ± 3↑*

Conscious, healthy chronically instrumented dogs. Data are mean ± SEM, showing absolute values predosing/baseline (PRE) and 3 hours (MAVA) postdosing; changes (vs PRE) are shown in *italics*. ↑, ↓: $P < 0.05$ vs PRE. Mavacamten: 1.5 mg/kg PO (n = 16).
EF: left ventricular ejection fraction; dP/dt_{max}: peak rate of left ventricular pressure increase during systole; CT: left-ventricular contraction time (pre-ejection period); Ees, and PRSW: elopes of the end-systolic elastance and preload-recruitable stroke work (respectively), derived from end-systolic/diastolic pressure-volume relationships during brief preload reductions; SV: left ventricular stroke-volume (calculated); MBP: mean arterial (systemic) pressure; HR: heart rate.

There was no appreciable change in LV end-diastolic filling pressure with the administration of MYK-461 despite of markedly decreasing contractility (e.g., EF and PRSW) and increasing end-diastolic ventricular volumes (EDV: +16%) (Fig. 29B), suggesting improved compliance and myocardial distensibility, a key determinant of diastolic cardiac filling (Table 18). This is shown as a downward or rightward shift of the LV pressure-volume relationships (Fig. 29A) indicating the ability of MYK-461 to

increase ventricular chamber dimensions and improve ventricular distensibility, which is consistent with its novel sarcomere-targeted mechanism of action.

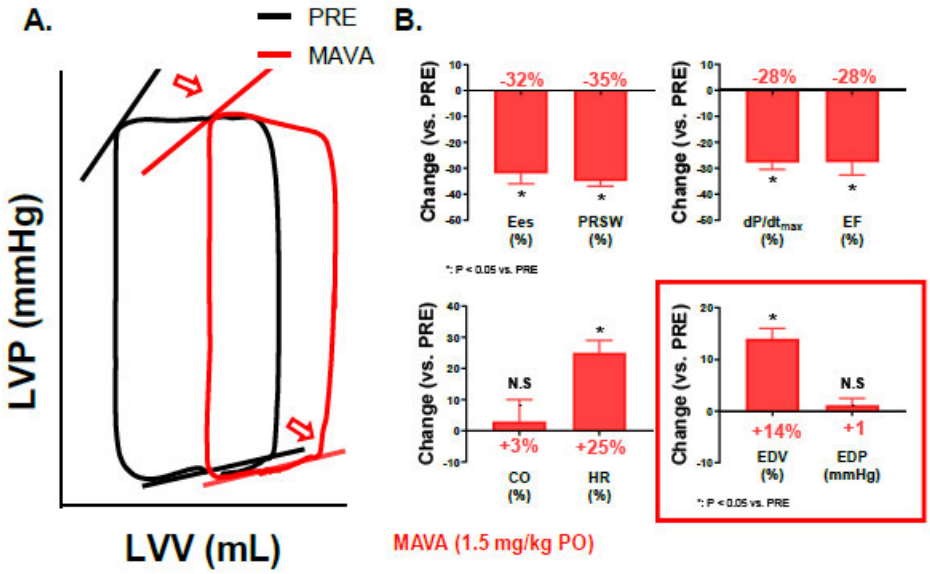


Figure 29. Acute hemodynamic and left ventricular effects of MYK-461 (MAVA) in conscious healthy dogs

In healthy conscious dogs, MYK-461 (MAVA, 1.5 mg/kg PO) produced a parallel rightward shift of the LV pressure-volume relationships (Panel A), decreasing indices of contractility while increasing end-diastolic volumes (EDV) but preserving end-diastolic pressures (EDP) and cardiac output (CO). dP/dtmax: peak rate of left ventricular pressure increases during systole; Ees and PRSW: end-systolic elastance and preload-recruitable stroke work (respectively) derived from pressure-volume relationships during brief preload reductions; EF: left ventricular ejection fraction; HR: heart rate; LVP: left ventricular pressure; LVV: left ventricular volume; PO: oral.

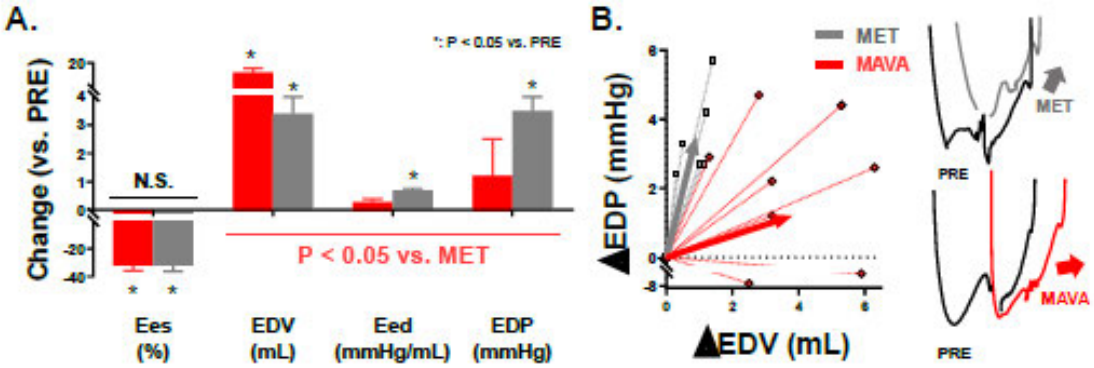
Data are mean ± SEM, showing relative changes (Panel B) vs predosing/baseline (PRE) at 3 h postdosing.

Beta-adrenergic receptor blockade with cardio-selective metoprolol (2 mg/kg PO) while inducing matched levels of negative inotropy caused different effects in LV filling (Table 19). At matched levels of cardio-depression (e.g., PRSW: -31%), metoprolol elevated EDP (6 to 10 mm Hg, $P < 0.05$) despite triggering smaller increases in preload (EDV: +3%), consistent with the normal physiological increase in ventricular elastance at end-diastole (Eed) with stretch (Eed: +37, $P < 0.05$) (Table 19 and Fig. 30A). Furthermore, metoprolol shifted the LV end-diastolic pressure-volume relationships right-upward, while MYK-461 shifted parallel, rightward (Fig. 30B). Both MYK-461 and metoprolol prolonged tau and decreased dP/dtmin, two load-dependent indices of active relaxation.

Table 19. Acute hemodynamic and LV effects of MYK-461 and metoprolol in dogs

	Mavacamten (1.5 mg/kg PO, n = 8)			Metoprolol (2 mg/kg PO, n = 6)		
	PRE	DOSE	*Change*	PRE	DOSE	*Change*
MBP (mmHg)	107 ± 6	109 ± 5	*+3 ± 4%*	103 ± 6	99 ± 5	*-4 ± 1↓*
PP (mmHg)	43 ± 3	31 ± 3	*-27 ± 4%↓*	39 ± 4	36 ± 4	*-8 ± 4%*
EDP (mmHg)	6 ± 1	7 ± 1	*+1 ± 1*	6 ± 0	10 ± 1	*+4 ± 1%↑*
ESP (mmHg)	122 ±6	114 ± 8	*-6 ± 3%*	115 ±6	111 ± 5	*-3 ± 1%*
dP/dt_{max} (mmHg/s)	3,190 ±231	2,342 ± 282	*-28 ± 3%↓*	3,088 ± 391	2,345 ± 189	*-22 ± 4%↓*
dP/dt_{min} (mmHg/s)	-2,944 ± 202	-2,297 ± 275	*-24 ± 5%↓*	-2,976 ± 229	-2,661 ± 189	*-10 ± 1%↓*
V_{max} (s^{-1})	61 ± 5	52 ± 4	*-13 ± 5%↓*	58 ± 2	47 ± 1	*-18 ±3%↓*
$tau_{½}$ (ms)	19.2 ± 1.2	24.6 ± 1.3	*+30 ± 5%↑*	17.1 ± 0.8	20.2 ± 0.9	*+19 ± 4%↑*
EDV (mL)	27.0 ± 0.7	30.8 ± 0.8	*+14 ± 2%↑*	27.7 ± 0.4	28.7 ± 0.4	*+3 ± 1%↑*
ESV (mL)	9.3 ± 0.7	16.4 ± 1.2	*+79 ± 14%↑*	9.5 ± 1.2	12.5 ± 1.2	*+35 ± 8%↑*
EF (%)	66 ± 2	47 ± 3	*-28 ± 5%↓*	66 ± 4	56 ± 4	*-14 ± 2%↓*
CO (L/min)	1.7 ± 0.1	1.7 ± 0.1	*3 ± 7%*	2.0 ± 0.1	1.5 ± 0.0	*-25 ± 3%↓*
Ea (mmHg/mL)	6.0 ± 0.3	7.6 ± 0.4	*28 ± 10%↑*	5.7 ± 0.4	6.3 ± 0.4	*+9 ± 3%↑*
Ees (mmHg/mL)	9.8 ± 0.8	6.6 ± 0.6	*-32 ± 4%↓*	9.4 ± 0.2	6.4 ± 0.4	*-32 ± 4%↓*
Eed (mmHg/mL)	2.0 ± 0.2	2.2 ± 0.3	*12 ± 4%*	1.8 ± 0.1	2.5 ± 0.1	*+37 ± 5%↑*
PRSW(*mmHg)	98 ± 7	64 ± 5	*-35 ± 2%↓*	101 ± 2	69 ± 3	*-31 ± 4%↓*

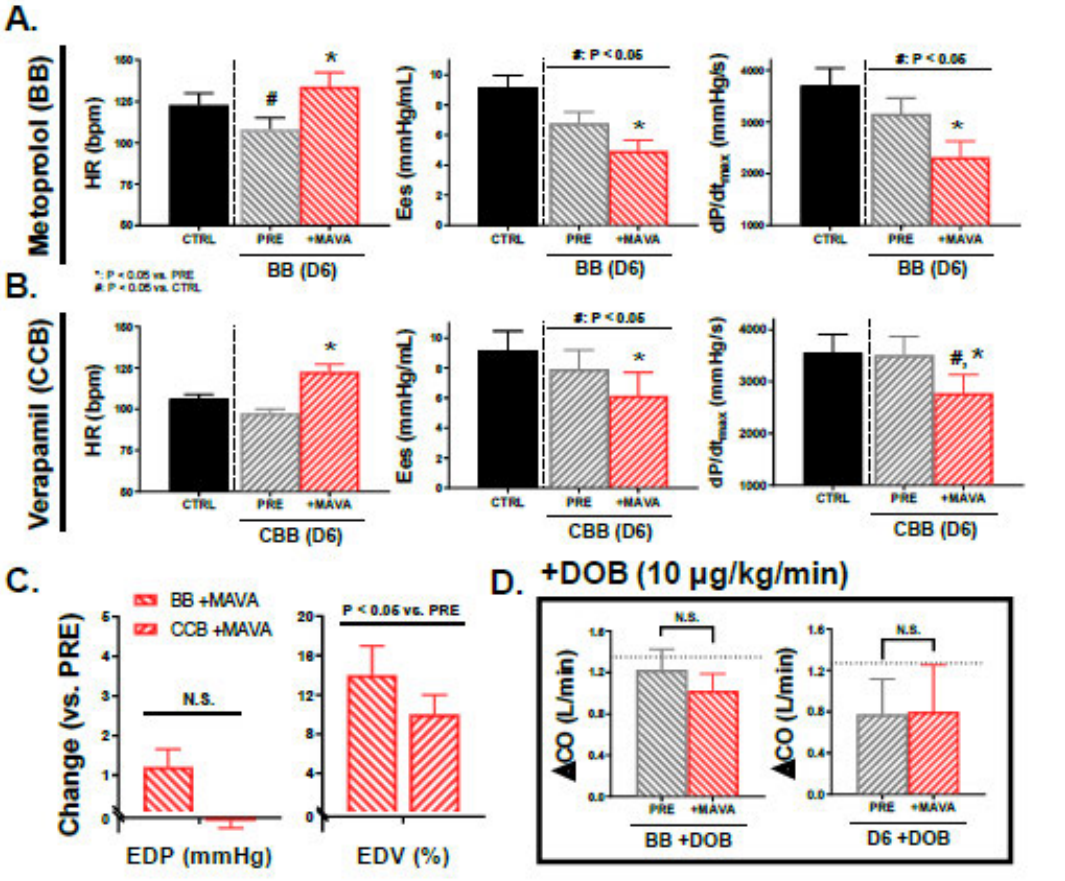
Conscious, healthy chronically instrumented dogs. Data are mean ± SEM, showing absolute values predosing/baseline (PRE) and 3 hours (DOSE) postdosing; changes (vs PRE) are shown in *italics*. ↑, ↓: $P < 0.05$ vs PRE. Mavacamten: 1.5 mg/kg PO (n = 8); Metoprolol: 2 mg/kg PO (n = 6)
CO: cardiac output (calculated); dP/dt_{max} and dP/dt_{min}: peak rates of left ventricular pressure increase/decrease during systole and diastole (respectively); Ea: estimated effective arterial elastance; EDP and ESP: left ventricular end-diastolic and end-systolic pressures (respectively); EDV and ESV: left ventricular end-diastolic and end-systolic volumes (respectively); Ees, Eed, and PRSW: end-systolic and end-diastolic elastances and preload-recruitable stroke work (respectively), derived from end-systolic/diastolic pressure-volume relationships during brief preload reductions; EF: left ventricular ejection fraction; MBP and PP: arterial (systemic) mean and calculated pulse pressure (respectively); PO: oral; $tau_{1/2}$: time-constant of left ventricular pressure decay (half-maximal); V_{max}: estimated maximal velocity of contractile element shortening.



Data are mean ± SEM, showing relative changes vs predosing/baseline (PRE) at 3 hours postdosing. *: $P < 0.05$ vs PRE. *Panel A*: At matched levels of negative inotropy (Ees reductions), mavacamten (MAVA, 1.5 mg/kg PO) and metoprolol (MET, 2 mg/kg PO) triggered significantly different effects in LV filling. *Panel B*: On average, mavacamten lead to a rightward (parallel) shift of the LV end-diastolic pressure-volume relationships, while β-AR blockade with metoprolol shifted it right-upward (representative curves in the insert).

Figure 30. Comparative left ventricular effects of MYK-461 (MAVA) and metoprolol in conscious dogs

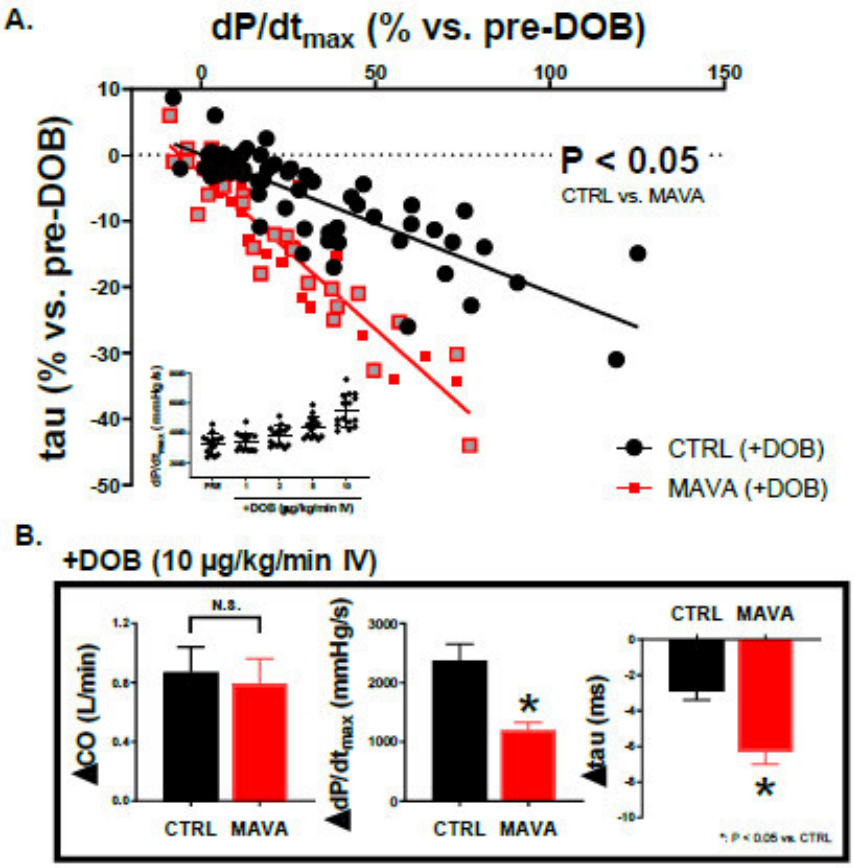
The novel pharmacological profile of MYK-491 (i.e., attenuated ventricular contractility with improved compliance) was confirmed with (mild) concomitant cardio-depression induced via either selective β-AR blockade with metoprolol or L-type Ca^{2+}-channel blockade with verapamil, the two therapies commonly used in patients with HCM. In these settings, MYK-491 led to additive (but preserved) negative inotropy (e.g., PRSW: -30% vs BB, and -28 vs CCB) and improved distensibility, increasing preload (EDV) but preserving EDP (Fig. 31C). Furthermore, according to the sponsor, the increase in heart rate noted at 1.5 mg MYK-491/kg (Table 18, Fig. 29) was not the result of baro-receptor reflex activation, as it was also observed in the setting of β-AR blockade (+24 ± 6%) and L-type CCB (+26 ± 6%) (Fig.31 A, B) suggesting β-AR activation remained operative under mavacamten treatment.



Data are mean ± SEM, showing absolute values prior to the onset of (PRE), as well as after sustained blockade (Day 6, D6), both before (PRE) and after (+3 hours, + MAVA) mavacamten administration. Single-dose mavacamten (MAVA, 1.5 mg/kg PO) given to dogs in the setting of (mild) cardio-depression induced via either selective β-AR (metoprolol, BB; *Panel A*) or L-type Ca^{2+}-channel blockade for 7 days (verapamil, CCB; *Panel B*) decreased indices of contractility and enhanced ventricular distensibility (*Panel C*); in all cases, mavacamten increased end-diastolic volumes (EDV) while preserving end-diastolic pressures (EDP). MAVA preserved cardiac output (CO)recruitment during β-AR stimulation with dobutamine (+DOB, 10 µg/kg/min IV) (*Panel D*).

Figure 31. Comparative left ventricular effects of MYK-461 (MAVA) and metoprolol in conscious dogs

The effects of MYK-461 on cardiac reserve were evaluated during β-AR challenge with dobutamine. Dobutamine increased both SV (+42 to +71% with MYK-461) and CO (+56 to +63% with MYK-461) before (i.e., control condition) and following acute MYK-461 treatment, despite the induced contractile depression. In spite of preserved recruitment of cardiac output with β-AR, MYK-461 blunted β–AR-induced increased dP/dtmax (+73% to +53%, P = 0.06) and Vmax (+71 % to +44%, P < 0.05), indicating contractile attenuation of β-AR stimulation (Fig. 32). Also, MYK-461 not only permitted systolic recruitment but also enhanced the β-AR stimulation–induced acceleration of relaxation (e.g., tau shortening) at any given dP/dtmax gain (Fig. 32), an observation consistent with the diastolic improvements noted above. Similar responses to β-AR stimulation were observed in the subsets of dogs under metoprolol or verapamil (Fig. 31).



Panel A: Acute β-AR stimulation (+DOB, dobutamine 1 to 10 µg/kg/min IV) dose-dependently increased the peak rate of left ventricular pressure development (dP/dt_{max}; inset, left) while concomitantly accelerating both the time constant of relaxation (tau) and the peak rate of pressure decay (dP/dt_{min}). Mavacamten treatment (MAVA, 1.5 mg/kg PO, red) blunted maximal dP/dt_{max} increases under dobutamine administration but enhanced the ability of this β-AR agonist to hasten relaxation (tau), thus preserving the recruitment of cardiac output (Panel B). β-AR: β-adrenoreceptor; Ca^{2+}: calcium ion; CO: cardiac output; CTRL: control; IV: intravenous; LVP: left ventricular pressure; LVV: left ventricular volume; PO: per os, oral; PRE: predose.

Figure 32. Acute effects of β-AR Stimulation (dobutamine) in in conscious dogs receiving MYK-461 (MAVA)

On the electrophysiology, MYK-461 at 1.5 mg/kg caused modest but significant downward shifts of the beat-to-beat QT versus RR relationship 3 h postdose, suggesting a potential to acutely accelerate repolarization. In this study, MYK-461 shortened both the absolute (mean) QT interval (215 vs 226 ms predose, $P < 0.05$) and the estimated QT interval at 1000 ms (QT1000: 225 vs 234 ms predose, $P < 0.05$) as derived (exponential fit) from the beat-to-beat relationship, despite preserved rate-corrected QT-intervals (Table 20). Also, MYK-461 was shown to prolong the electromechanical window (EMw: 161 vs 113 ms predose, $P < 0.05$), defined as the time interval between the end of electrical repolarization (Tend) and the onset of mechanical filling (Pmin) in the ventricle (Table 20); shortening of the EMw has been associated with known torsadogens, thus supporting a low proarrhythmic potential for MYK-461. No QT changes were noted up to 24 hours postdosing, suggesting preserved cardiac conduction.

Table 20. Acute electrocardiographic effects of MYK-461 in dogs

	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTcF (msc)	QT_{1000} (ms)	EMw (ms)	dP/dt_{max} (mmHg/s)
PRE	91 ± 8	89 ± 4	43 ± 2	226 ± 9	261 ± 4	234 ± 7	113 ± 6	3189 ± 431
3 h	106 ± 7↑	88 ± 4	43 ± 2	215 ± 7↓	261 ± 5	225 ± 7↓	161 ± 15↑	2331 ± 298↓
24 h	108 ± 10↑	89 ± 4	41 ± 1	214 ± 7	259 ± 3	226 ± 6	133 ± 7↑	2766 ± 258

dP/dt_{max}: peak rate of left ventricular pressure increase during systole; EMw: electromechanical window, or time interval between the end of electrical repolarization (Tend) and the onset of mechanical filling (Pmin) in the left ventricle; HR: heart rate; MAVA: mavacamten; PO: oral; PR, QRS, and QT: duration of the electrocardiographic intervals reflecting atrio-ventricular (PR) and ventricular conduction (QRS), as well as repolarization (QT); QT_{1000}: estimated QT interval duration at an RR interval of 1000 ms, from an exponential fit to the beat-to-beat QT and RR values; QTcF: rate-corrected (Fridericia) QT interval.
↑, ↓: $P < 0.05$ vs PRE.
Dosing: MAVA, 1.5 mg/kg PO; n = 7; subjects were conscious and chronically instrumented. Data are mean ± SEM, showing absolute value both predosing/baseline (PRE) as well as at 3 hours and 24 hours postdosing; changes (from PRE) are shown in *italics*.

4.2 Secondary Pharmacology

1. Besides confirming on-target selectivity, MYK-461 was evaluated for its secondary pharmacology activity against a panel of kinase assays designed for broad profiling across the human kinome, as well as multiple primary molecular targets including enzymes and binding receptors (study #NC14-0009, study date, May 16, 2013). MYK-461 was tested at 10 µM. No noteworthy results were noted (≥ 50% inhibition or stimulation).

2. MYK-461 was tested for cytotoxic effects via luminescence (at 0.1, 0.3, 1, 3, 10, and 30 µM for 48 hours) in 13 different cell lines (Report NC14-0007, dated September 23, 2013). Cells studied were: human foreskin fibroblasts (BJ), rat hepatoma cells (H-4-II-E), human embryonic kidney cells (HEK-293), human hepatocellular carcinoma cells (HepG2), human renal glomeruli mesangial cells (HK2), human vascular endothelial cells (HUV-EC-C), human T cells leukemia (Jurkat), human lung fibroblasts (MRC-5), mouse neuroblastoma cells (Neuro-2a), mouse embryonic fibroblasts (NIH/3T3), rat kidney proximal tubule cells (NRK-52E), human neuroblastoma cells (SH-SY5Y), and human neuroblastoma cells (SK-S-SH). MYK-461 did not show any cytotoxicity in all 13 cell lines at test concentrations between 0.1 and 30 µM with 48 h incubation.

3. In a separate study (Report NC14-0008, dated July 8, 2013), cytotoxicity of MYK-461 (up to 10 µM for 72 hours) was assessed in HepG2 (human hepatoma) cells. No evidence of cytotoxicity was observed, as cell counts, nuclear area, DNA structure, mitochondrial mass, and membrane potential were unchanged relative to vehicle control, showing no cytochrome C release. However, a small increase in cell membrane permeability was observed with (estimated) minimum effective and half-maximal (AC50) concentrations of 4.08 µM and >10.0 µM, respectively.

4.3 Safety Pharmacology

The following table summarizes the core safety pharmacology studies performed to determine neurobehavioral, cardiovascular and respiratory effects of MYK-461.

Type of Study	Section/ Study #	Species/ Media	Route	Dose (mg/ kg) or bath conc	Findings
1. Inhibition of hERG channel (GLP study)	4.3.1 NC-14-0061	hERG-expressing HEK293 cells	*In vitro*	10 and 60 µM	Weak inhibition, 5 and 10% at 10 and 60 µM, respectively.
2. Cardio-vascular telemetry, acute (GLP study)	4.3.2 NC-15-0007	Beagle dog	Oral	Single doses of 1, 3, 10	Marked dose-related decrease in BP and compensatory increase in HR at 3 and 10 mg/kg, lasted up to 1 week. Increase in QT/QTc interval by 22 msec at 10 mg/kg.
3. Cardio-vascular telemetry, repeat dose (Non-GLP study)	4.3.3 NC-20-0060	Dog	Oral	1.5 mg/kg twice on Day 1, followed by 14 doses of 0.3 mg/kg/day	Marked reductions in fractional shortening, both QT and QTcF prolonged that correlated to increase in EDV.
3. Respiratory (GLP study)	4.3.2 NC-15-0007	Beagle dog	Oral	Single doses of 1, 3, 10	Increases in respiratory rate and decrease in tidal volume at 10 mg/kg
4. CNS (GLP study)	4.3.4 NC-15-0006	Rat	Oral	Single doses of 1, 3, 10	No treatment-related effect on CNS except for a decrease (-1^{o}C) in body temperature at 10 mg/kg
5. Cardiac ion channels	4.3.5 NC-19-0035	HEK293 cells	*In vitro*	30 µM	Inhibition ~13% maximum for all ion channels
6. Purkinje fibers, action potential	4.3.6 NC-19-0036-0037	Rabbit	*In vitro*	30 µM	No effect
7. Electrophysiol	4.3.7 NC-20-0061	hVentricular myocytes, HEK293 cells	*In vitro,* In silico	0.3 to 30 µM	Multichannel mild to moderate effect, APD shortened

4.3.1. Effect of MYK-461 on hERG channels expressed in HEK293 cells

This GLP study (Study #131217.NZQ; Report #NC-14-0061) was conducted at (b) (4) between July 30 and August 15, 2014. The study report and its QA statement are not signed. The objective of the study was to evaluate the *in vitro* effects of MYK-461 on human ether-a-go-go-related gene-encoded channels, which are the pore forming subunits of I_{Kr}. The I_{Kr} current is critical for repolarization of the cardiac action potential. Any blockade *in vivo* may cause prolongation of the QT interval.

Methods

For the assay, hERG potassium channels were expressed in a human embryonic kidney (HEK293) cell line. A stock solution of MYK-461 (lot #6, purity 99.17%) was prepared in DMSO and varying concentrations were prepared fresh on the day of use in HEPES buffer (final DMSO concentration, 0.3% v/v). Samples for homogeneity determination were aliquoted at the beginning of testing. For concentration analyses, samples were aliquoted from the outflow apparatus. Positive control (terfenadine) and reference substance (E-4031) were prepared in DMSO and aliquoted for individual use at the beginning of study. Homogeneity, concentration and stability analyses were not performed on these two compounds. Initially, for concentration range determination, two concentrations (n=4 cells) of test substance were selected to evaluate concentration-response relationship and the solubility limitations in the assay buffer. Vehicle control was tested in 3 cells. Duration of application was maximum 5.5 min.

Cells were mounted in a recording chamber and superfused with HEPES buffer at pH 7.4 (at 33 to 35ºC). The hERG tail current was recorded using the whole-cell patch clamp technique. Onset and steady state inhibition of peak tail current was measured in a vehicle control and MYK-461 solutions (at concentrations 10 and 60 µM). Each recording ended with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM) to assess the contribution of endogenous currents. The remaining uninhibited current was subtracted off-line digitally from the data to determine the potency of the test substance for hERG inhibition.

Results

At high concentrations (≥10 µM), MYK-461 was stable at room temperature (15 – 30ºC) for a period of at least 28 hours in HEPES buffer + 0.3% DMSO. However, for 0.1 µM concentration evaluated, MYK-461 was stable at room temperature (15 – 30ºC) for a period of at least 6 hours. The achieved concentrations for the dose formulations of

MYK-461 were 10.3 and 60.8 µM for 10 and 60 µM, respectively; these results were within ± 15% of nominal, thereby meeting the acceptance criteria.

MYK-461 reduced the amplitude of the outward tail currents by 9.6 ± 0.6% at 60 µM. At the low concentration, 10 µM, MYK-461 inhibited hERG current by 5.0 ± 0.6% (Mean ± SEM) (Fig. 33). Higher concentrations were not evaluated because of solubility limitations of the test substance in the vehicle. Although IC_{50} was not calculated, on extrapolation it is greater than 60 µM. The positive control, terfenadine, at 60 nM produced 80.0 ± 2.8% mean inhibition of hERG potassium current.

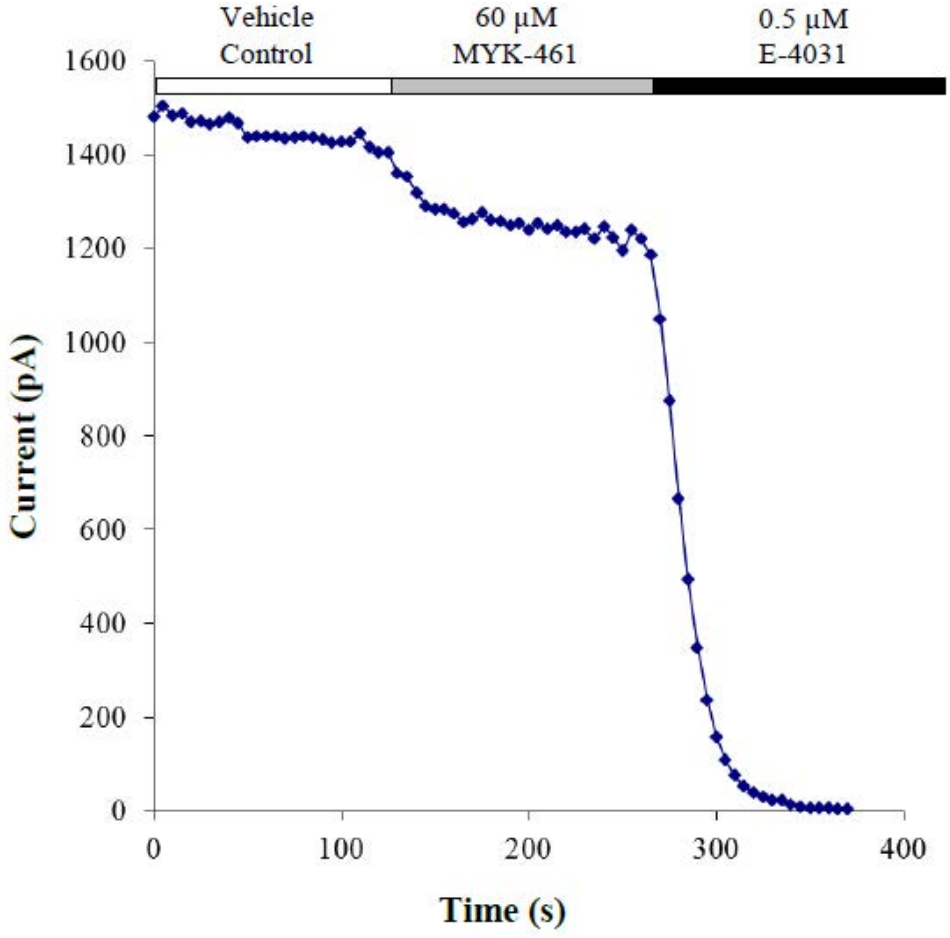


Figure 33. Typical time course of the effect of MYK-461 on hERG current from transfected HEK 293 cells. Peak current amplitude during application of vehicle control, test article and reference substance is illustrated. The horizontal bars indicate the vehicle control, test article concentration and E-4031.

4.3.2. Effect of single dose of MYK-461 on cardiovascular and respiration in telemetered dogs

A GLP study (study #14-6827, Report #NC-15-0007) was conducted at (b) (4) between March 19 and May 29, 2014. The objective of the study was to evaluate the effects of single oral doses of MYK-461 on cardiovascular and respiratory effects in conscious male dogs. The study was designed with reference to the ICH-S7A and S7B guidelines for safety pharmacology studies for human pharmaceuticals.

Methods

The experiments were performed on 16 male non-naïve (previously instrumented with transmitter implants) beagle dogs weighing between 6.8 and 12.1 kg (12 and 30 months of age) at initiation of dosing. Latin square study design was not used because of long half-life of the drug in dogs (approximately 5 days). The study consists of 4 groups of 4 telemetered dogs/group, one for the vehicle, 2, 3 and 4 for the MYK-461 dosing regimen 1, 3 or 10 mg/kg, respectively. Test substance (lot #400-13-01-53) was administered orally by gavage (10 ml/kg). Prior to dosing, telemetry data was collected continuously for at least 2 hr to determine the baseline. Following dosing, data was collected for the next 24 h. Additionally, 24 h of continuous recording was done on days 7, 14, 21 and 28 postdose. Blood samples for the determination of plasma concentrations of MYK-461 and MYK-460 (enantiomer) were collected from each non-fasted animal on days 1 (at 24 h), 9, 16, 23 and 30. Also, upon completion of the telemetry data collection, animals receiving 10 mg/kg received an additional dose of 10 mg/kg and blood samples were collected 0.5, 1, 2, 4, 8, and 24 h post dose.

After completion of first phase, 4 control animals of group 1 were dosed with 10 mg MYK-461/kg again to collect intermediate time points to characterize the time course of recovery. Following drug administration, telemetry data were collected continuously for 4 days. In addition, 24 hr continuous recording was performed on day 7 post dose. For TK analyses, blood samples were obtained from jugular or cephalic venipuncture from this group of non-fasted animals at 0.5, 1, 2, 4, 8 and 24 h post dose.

Animals were housed individually in cages and were fed (400 gm/day) every 4 to 5 hr daily and had unlimited drinking water. All animals had previously been surgically implanted with telemetry transducers to record blood pressure, heart rate, ECG and body temperature. MYK-461 was formulated in 0.5% methylcellulose in distilled water. The formulation was stable when stored at room temperature for 10 days. Prior to initiation of dosing, concentration and homogeneity of the preparation were confirmed.

Dose levels were selected based on the results of a single dose tolerability study in two dogs in which 1.5, 4.5 and 7 mg/kg were tolerated. Acute administration of 30 mg/kg resulted in moribund euthanasia of both dogs. In a 28-day exploratory oral toxicity study, 0.4 and 1.3 mg/kg/day was well tolerated but not 4 mg/kg/day. Due to mortality and excessive toxicity, the group receiving 4 mg/kg/day was terminated on day 15.

All animals were observed twice daily for mortality, injury and clinical signs. Body weights were recorded predose phase and prior to each dose and weekly during the study. Cardiovascular and respiratory telemetry parameters were examined each day before operation. CV parameters included arterial blood pressure, heart rate and ECG (PR, RR, QRS, QT intervals). Respiratory parameters included tidal volume, respiratory rate and minute volume. Animals were returned to the colony after completion of the study. Individual regressions of QT interval on heart rate were performed for each dog. QT corrected for heart rate (QTcR) was calculated using the correction formula by Ollerstam A. et al 2007 (*Pharmacol Toxicol Methods 55(1): 35-48*):

$QTc = QT + \beta(HRM-HR)$
Where ß is calculated as the slope of QT versus HR and HRM is the mean heart rate, both of which are derived from the data extracted at the stated time points.

Results

All formulation samples were within the acceptable concentration range (94.9 to 104.7% of the nominal concentrations). No mortality was observed. Clinical signs were restricted to animals receiving 10 mg/kg and that included vomiting, decreased activity, rapid breathing in one animal and decreased food consumption in all 4 animals. No effect was seen on body weight.

Following the oral administration of MYK-461 at doses of 3 or 10 mg/kg, dose-related decreases in systolic (-23 mm Hg, 15% and -39 mm Hg, 26% from the pre dose, respectively, peak 3-4 h post dose) and pulse pressure were noted up to week 1 post dose. There were concomitant compensatory dose-related increases in heart rate (+42 bpm, 47% and +119 bpm, 123% from the predose at 3 and 10 mg/kg, respectively, at 2 hr post dose) up to day 4 (Fig. 34). An oral dose of 1 mg/kg did not produce an effect on arterial blood pressure or heart rate.

A single oral dose of 10 mg/kg increased QTc interval from predose of 18 msec at 2 to 4 hr post dose, reaching a peak of 22 msec at 24 hr postdose and lasted up to day 8 (a peak of 30 msec, Fig. 35) although plasma concentration of drug was at its lowest point (188 ng/ml). The time periods of increased QTc interval were concurrent with increased

heart rate and decreased QT interval on Day 1 but not Day 8. Additionally, a single dose of 3 or 10 mg/kg produced higher incidences of ventricular escape beats in one dog at each dose level up to week 3. A higher incidence in the number of ectopic activities of ventricular origin was noted in 5 of 8 high dose animals up to week 3. According to the sponsor the risk for the test article producing arrhythmia of either torsadogenic or non-torsadogenic origin are minimal. Maximum plasma exposure at 10 mg/kg at 2 hr was an average 729 ng/ml (Table 21) where peak cardiovascular changes were noted.

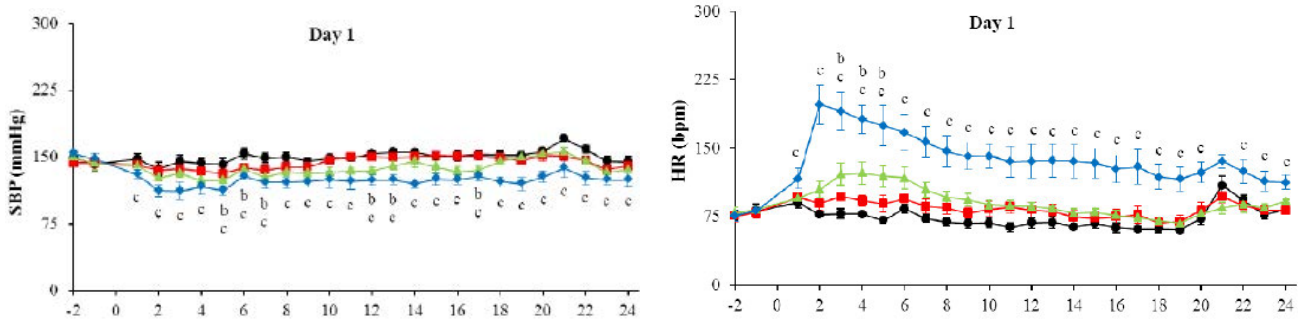


Figure 34. The effect of MYK-461 on systolic BP (mm Hg) (Left) and heart rate (bpm) (Right).
Black: Control, Red: 1 mg/kg, Green: 3 mg/kg, Blue: 10 mg/kg. An 'a', 'b' or 'c' indicates that the mean value of the 1, 3 or 10 mg/kg group is statistically different from the mean value of the control group.

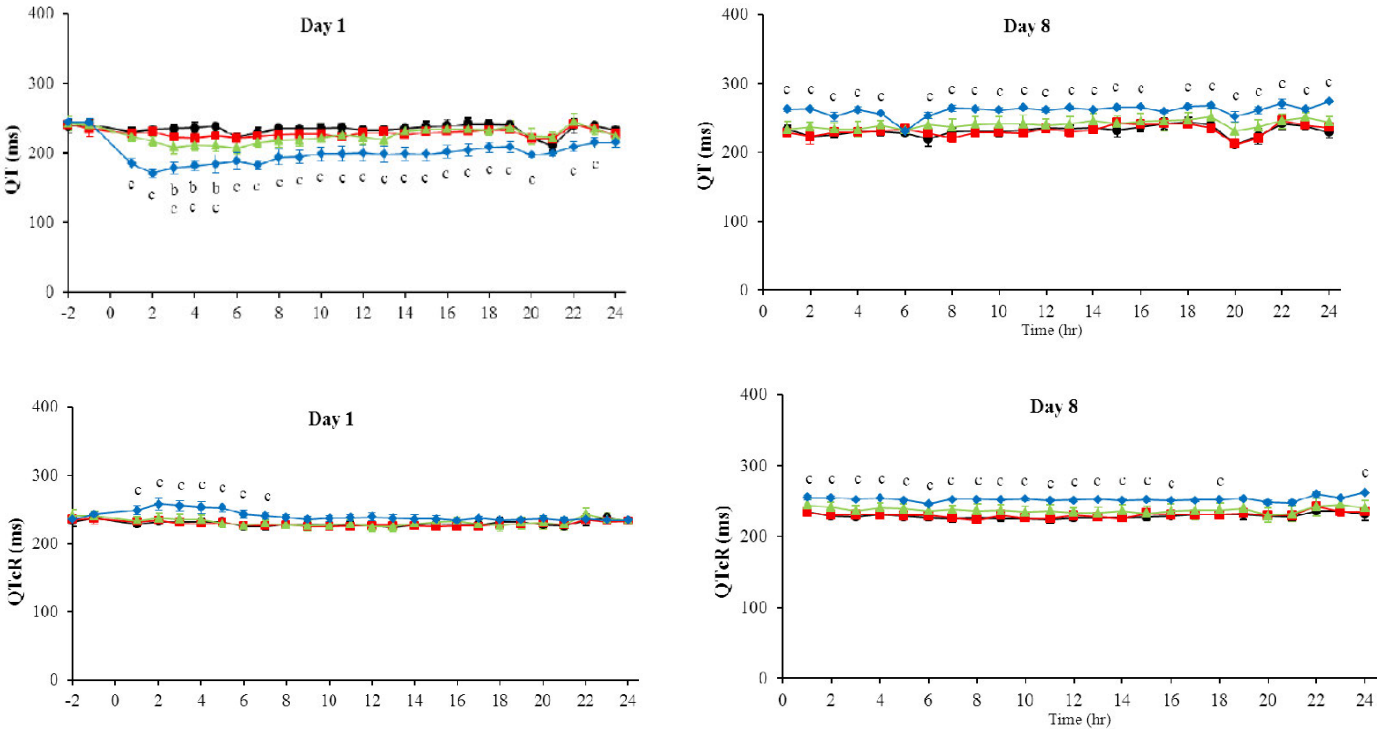


Figure 35. The effect of MYK-461 on QT interval (top) and heart rate corrected QT (2nd panel) (ms).
Black: Control, Red: 1 mg/kg, Green: 3 mg/kg, Blue: 10 mg/kg. An 'a', 'b' or 'c' indicates that the mean value of the 1, 3 or 10 mg/kg group, respectively, is statistically different from the mean value of the vehicle control group.

All animals were exposed to quantifiable concentrations of active drug. A dose-dependent but non-linear increase in plasma concentrations of MYK-461 was noted over the dose range of 1 to 10 mg administered. An increase in the dose of MYK-461 above 3 mg/kg increased systemic exposure less than proportionately to the dose. Plasma concentrations of MYK-461 were still quantifiable on day 30 postdose at all dose levels and in all animals (Table 21 supplement). Inter-animal variability at any time point or interval day was very high. The mean enantiomer ratio was 0.0087, indicating a limited inter-conversion of MYK-461 to MYK-460 (enantiomer) (Table 21).

Table 21. Pharmacokinetic parameters of MYK-461 and MYK-460 following a single oral dose of 10 mg/kg MYK-461 upon completion of the telemetry data collection (after day 30).

MYK-461					
Dog ID number	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24} (ng.h/mL)	AUC_{0-24}/D (ng.h/mL)/(mg/kg)
5190	850	2	652	14600	1460
7047	642	2	355	10500	1050
7071	608	1	603	12500	1250
9796	814	2	807	16300	1630
Mean	729	2[a]	604	13500	1350
SD	121		188	2500	250
MYK-460					
Dog ID number	C_{max} (ng/mL)	T_{max} (hours)	C_{24} (ng/mL)	AUC_{0-24} (ng.h/mL)	AUC_{0-24}/D (ng.h/mL)/(mg/kg)
5190	13.7	2	1.01	143	14.3
7047	9.93	2	BLQ	88.7	8.87
7071	13.0	2	2.72	177	17.7
9796	11.9	2	1.38	141	14.1
Mean	12.1	2[a]	1.28	137	13.7
SD	1.6		1.12	36	3.6

[a] Median

Table 21 Supplement. Mean plasma concentrations (ng/ml) of MYK-461 following a single oral (gavage) administration of MYK-461 to dogs (n = 4) at dose levels of 1, 3 or 10 mg/kg on day 1. Plasma concentration determined weekly until day 30

Day	Dose (mg/kg)		
	1	**3**	**10**
1	75.1	220	540
9	24.8	74.9	188
16	8.3	26	70.2
23	3.01	9.39	27
30	1.15	2.98	8.84

There was no effect on body temperature. Regarding respiration, a single oral dose of 10 mg/kg produced an increase in respiratory rate (1 to 18 h post dose, maximum increase noted at 3 h post dose), a decrease in tidal volume (2 to 24 h post dose) with no change in minute volume on day 1.

4.3.3. Effects of repeat doses of MYK-461 on cardiovascular performance and ECG in telemetered dogs

A non-GLP study (Study No. 01083001, Report #NC-20-0060) was conducted at (b) (4) between March 20 and April 4, 2018. The objective of the study was to evaluate the effects of repeat oral doses of MYK-461 on hemodynamics, cardiac performance and ECG in conscious telemetered male dogs.

Methods

The experiments were performed on 4 healthy male non-naïve (previously instrumented with transmitter implants) beagle dogs weighing between 6 and 13 kg (6 months of age) at initiation of dosing. On day 1, 4 dogs received test vehicle. On day 2, the same 4 dogs received 2 doses (capsule) of MYK-461 (Lot #5) at 1.5 mg/kg (loading dose), administered approximately 8 h apart. On day 3, all 4 animals in group 3 received a daily dose of 0.3 mg/kg/day for the next 14 days (Table 22). For exposure determination, blood samples were collected from all animals prior to dosing and on days 1, 3, 7, 14, 17, 32 and 42 post dose. Left ventricular pressure waveforms (LV end diastolic pressure, dP/dtmax, dP/dtmin), arterial blood pressure (systolic, diastolic, and mean), pulse pressure, heart rate, electrocardiographic (ECG) waveforms, and body temperature were collected continuously for approximately 24 hours beginning with 1 hr before administration of vehicle/MYK-461, and on days 1, 2, 4, 8, 11 and 15. Ventricular function and structure were evaluated via transthoracic echocardiography both prior to dosing (i.e., Day -1) and on study days 7 and 14 at approximately 3 h post-dose.

Table 22. Study design

Group	Day	Treatment	Dosing Route	Dose Level (mg/kg)	Doses/day	Number of Males[a]
1	1	Vehicle	Capsule	0	1	4
2	2	MYK-461	Capsule	1.5	2	4
3	3-15	MYK-461	Capsule	0.3	1	4

a. The same 4 males will be used for each treatment period.

Results

The dosing regimen achieved sustained exposures over the 14-day study period; the total MYK-461 exposures (in plasma) were 374.5, 374, 415 ± 48, 426 ± 54, 423.5, 95.5 and 17 ng/mL on Days 1, 3, 7, 14, 17, 32 and 46, respectively. Only the MYK-2241 and

MYK-1096 metabolites were detected, both at negligible (<2% of parent) levels (e.g., on Day 15, 1.3 ± 0.1 ng/mL and 4.6 ± 0.4 ng/mL, respectively).

MYK-461 showed marked reductions in fractional shortening (on Day 15, EF: -26% and dP/dtmax: -22%, both P < 0.05 vs before treatment), increased LV end-diastolic volumes (EDV: +24%, P < 0.05). Concomitantly, upward shifts of the beat-to-beat QT versus RR relationship and QT prolongation were observed with QTcF prolongations reaching +19 ± 2 ms (274 ± 8 vs 255 ± 6 ms predose, P < 0.05) at the end of the study (Day 15) (Table 23). QTcF prolongations are related (non-linearly) to EDV increases (R2 = 0.6715). According to the sponsor, QT prolongation was driven primarily to lengthening of the JTp (on Day 15, +19 ± 4 ms, 162 ± 5 vs 144 ± 4 ms at predose, P < 0.05), reflecting early repolarization (J-wave) changes, with negligible changes observed in the terminal portion of repolarization (TPE, on Day 15: +3 ± 2 ms, 43.0 ± 2.5 vs 40.4 ± 3.0 ms predose, P >0.05) (Fig. 36). This suggest preserved transmural repolarization and unchanged late repolarizing (e.g., hERG) currents. No other notable electrocardiographic changes were observed postdosing, as both QRS and PR intervals as well as heart rate and the length of the electromechanical window remained unchanged.

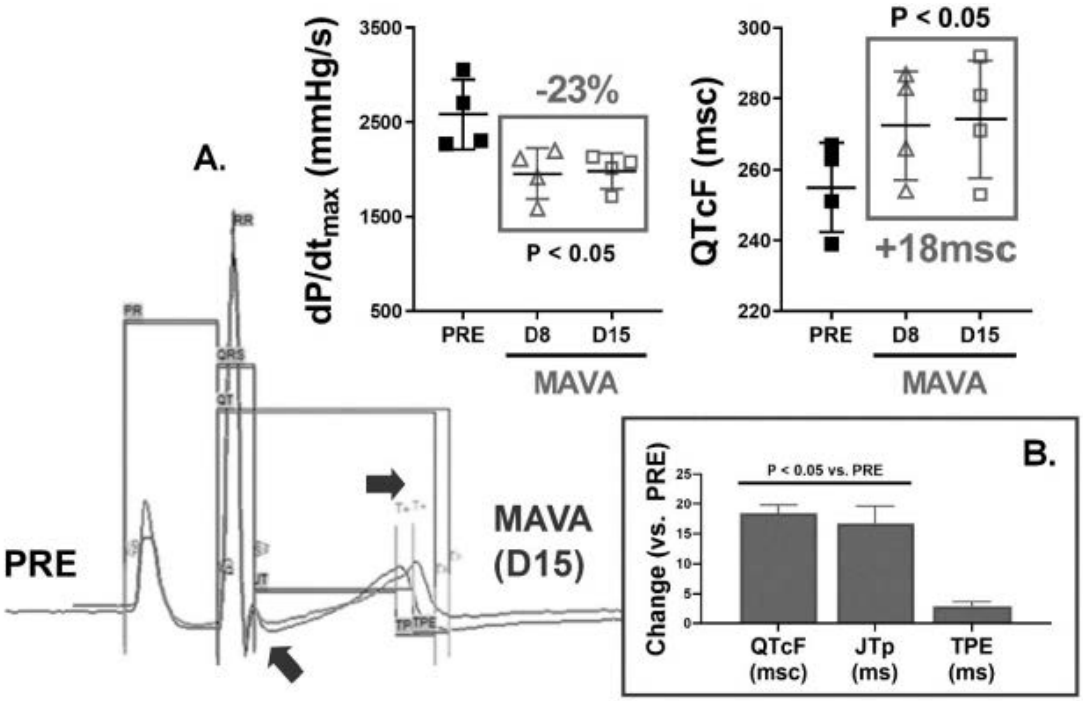
Table 23. Chronic electrocardiographic and mechanical effects of MYK-461 in healthy dogs (14-day repeat-dose study)

	Mechanical			Electrocardiographic							
	EF (%)	EDV (mL)	dP/dt_{max} (mmHg/s)	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTcF (ms)	JTp (ms)	Tpe (ms)	EMw (ms)
PRE	71 ± 2	40.5 ± 6.4	2,584 ± 185	77 ± 3	98 ± 4	45 ± 3	230 ± 6	255 ± 6	144 ± 4	40 ± 3	84 ± 5
Day 8	57 ± 2	46.5 ± 6.9	1,957 ± 136	80 ± 2	90 ± 3	43 ± 2	243 ± 8	273 ± 8	159 ± 7	43 ± 3	88 ± 5
Change	*-21 ± 4%↓*	*+15 ± 1%↑*	*-24 ± 4%↓*	*+5 ± 5%*	*-8 ± 3%*	*-4 ± 2%*	*+5 ± 2%*	*+17 ± 2↑*	*+15 ± 5*	*+3 ± 1*	*+6 ± 2%*
Day 15	53 ± 3	49.7 ± 6.4	1,987 ± 93	78 ± 3	91 ± 4	43 ± 2	247 ± 9	274 ± 8	162 ± 5	43 ± 2	89 ± 6
Change	*-26 ± 2%↓*	*+24 ± 3%↑*	*-22 ± 4%↓*	*+3 ± 7%*	*-7 ± 3%*	*-3 ± 2%*	*+7 ± 2%*	*+19 ± 2↑*	*+19 ± 4↑*	*+3 ± 2*	*+7 ± 4%*

dP/dt_{max}: peak rate of left ventricular pressure increase during systole; EDV: left ventricular end-diastolic volume; EF: left ventricular ejection fraction; EMw: electromechanical window, or time interval between the end of electrical repolarization (Tend) and the onset of mechanical filling (Pmin) in the left ventricle; HR: heart rate; JTp and Tpe: early (JTp, interval from the J-point to the peak of the T-wave) and terminal (Tpe, interval from the peak of the T-wave to its end); PO: oral; PR, QRS, and QT: duration of the electrocardiographic intervals reflecting atrioventricular (PR) and ventricular conduction (QRS), as well as repolarization (QT); QTcF: rate-corrected (Fridericia) QT interval.
↑, ↓: $P < 0.05$ vs PRE.
Dosing: 1.5 mg/kg twice daily on Day 1, 0.3 mg/kg/day on Days 2 to 15 PO; n = 4. Subjects were healthy, conscious, and continuously telemetered. Data are mean ± SEM, showing absolute value both predosing/baseline (PRE) as well as after 7 (Day 8) and 14 days (Day 15) of treatment; changes (from PRE) are shown in *italics*.
Total mavacamten exposures (in plasma): 415 ± 48 (Day 8) and 426 ± 54 ng/mL (Day 15).

According to the sponsor, these in vivo observations in healthy ventricles describe a biphasic ECG profile for MYK-461, characterized by preserved or shortened QT intervals upon acute administration (up to 24 hours) as well as by a late-onset QT/JTp prolongation during sustained exposure. This delayed QT prolongation, though likely hERG independent and with a low torsadogenic or proarrhythmic risk, occurs as adaptive mechano-electrical remodeling due to sustained concomitant functional and structural effects but could also be indicative of a direct effect of MYK-461 (or a

metabolite) on ionic homeostasis, currents, or channel trafficking. As such, multiple in vitro electrophysiological studies were performed to elucidate both the specific mechanism(s) of the QT behavior and its potential torsadogenic risk (see section 10.2 for additional studies on proarrhythmic risk with MYK-461).



dP/dt_{max}: peak rate of left ventricular pressure increases during systole; JTp and Tpe: early (JTp, interval from the J-point to the peak of the T-wave) and terminal (TPE, interval from the peak of the T-wave to its end); PO: oral; PRE: predose.

Panels A: Sustained mavacamten exposure (MAVA for 14 days; 1.5 mg/kg twice daily on Day 1, 0.3 mg/kg/day on Days 2 to 15) in healthy beagle dogs triggered marked function depression (eg, dP/dt_{max}) that was accompanied by rate-corrected QT interval prolongations (Fridericia, QTcF). *Panel B:* As shown in the representative electrocardiogram, QT changes were driven primarily by lengthening of the JTp, with negligible changes observed in the terminal portion of repolarization (TPE).

Figure 36. Chronic electrocardiographic and mechanical effects of sustained MYK-461 exposure in healthy dogs

4.3.4. Effect of MYK-461 on CNS function in male rats

A GLP study (study #14-6826, Report #NC-15-0006) was conducted at (b) (4) between April 7, 2014 and March 4, 2015. The objective of the study was to evaluate the effects of single oral doses of MYK-461 on CNS in male rats.

Methods

The experiments were performed on 32 male Sprague-Dawley CD rats weighing between 254 and 329 gm (approximately 10 weeks of age) that were assigned to 4 groups (n = 8/group). Animals were housed 2 or 3 per cage before dosing but single housed the day before dosing/ CNS assessment and remained single-housed through the 24-hr evaluation. Food and water supplied *ad libitum*. MYK-461 (lot #400-13-01-53) was formulated in 0.5% methylcellulose in distilled water. The formulation was stable when stored at room temperature for 10 days. Prior to initiation of dosing, concentration and homogeneity of the preparation were confirmed. A single dose of vehicle (5 ml/kg) or 1, 3 or 10 mg MYK-461 was administered orally by gavage.

Dose levels were selected based on the results of a previous single dose tolerability study at 4 and 12 mg/kg. The high dose resulted in an 8% reduction in body weight gain. MYK-461 was further evaluated in an exploratory 14-day toxicity study in which 3 and 10 mg/kg/day doses caused centrilobular hepatocellular vacuolation. The high dose, 10 mg/kg/day, was not tolerated for 14 days and resulted in adverse cardiac and hepatic effects.

Observations

Rats were observed twice daily for signs of toxic or pharmacologic effects. Gross neurobehavioral evaluations were performed pretest, and 2 and 24 hr post dose. A functional observational battery (FOB) was performed on all animals blindly. The basic studies included: posture (sitting, standing and sleeping), palpebral (eyelids) closure, vocalizations, motor movements, ease of removal, reactivity to handling, lacrimation, salivation and coat. Advanced studies included open field evaluations such as gait and posture (ataxia, hind limbs and forelimbs drag, walks on tip toes, hunched body position, body drags or flattened), locomotion (any impairment in movement), arousal (alert, sluggish or stupor), piloerection, exophthalmia, motor movements, reflex assessments (visual, audition, pinna reflex, proprioception, pain perception, pupil response, righting reflex) and grip strength. Body temperature was also recorded.

Following completion of the monitoring period, all animals were euthanized, and the carcasses discarded without further examination.

Results

All formulation samples were within the acceptable concentration range (99.5 to 104.2% of nominal). There were no deaths or clinical signs noted in the study.

Oral administration of MYK-461 at dose levels up to 10 mg/kg exhibited no marked behavioral and physiological changes at any of the time points evaluated. Low arousal levels (slight to moderate) were noted at all dose levels at 2 and 24 hr post dose. A few animals in the control group also exhibited these signs. Additionally, the response was not dose-dependent and thus, the sponsor concludes that the observations of low arousal level at 10 mg/kg were attributed to normal variability. Also, there were no MYK-461-induced changes in forelimb/hindlimb grip strength as well as locomotion activity relative to vehicle control at the highest dose tested. The studies demonstrate that MYK-461 has no impact on skeletal muscle performance or function in vivo at exposures triggering marked reductions in LV function.

A statistically significant decrease in mean body temperature was noted in animals receiving 10 mg/kg (-1.0ºC) relative to control (-0.4ºC) at 2 and 24 h post dose measurements. This suggests that the decrease in temperature is test substance related.

4.3.5. Effect of MYK-461 and its metabolite on cardiac ion channel currents expressed in HEK293 cells

This non-GLP study (Study # 170601.NZQ; Report #NC-19-0035) was conducted at (b) (4) between July 10 and August 07, 2017. The objective of the study was to evaluate the *in vitro* effects of MYK-461 and MYK-1078, (primary metabolite of MYK-461) on calcium, potassium and sodium channels expressed in mammalian cells.

Methods

The study examined the in vitro effects of MYK-461 and MYK-1078 on the following cardiac ion channels expressed in HEK293 cells.

1. Cloned L-type calcium channels (hCav1.2, encoded by the human CACNA1C gene and co-expressed with the β2 subunit, encoded by the human CACNB2 gene and the

α2δ1 subunit encoded by the human CACNA2D1 gene in CHO cells), responsible for $I_{Ca,L}$, high threshold calcium current.
2. Cloned T-type calcium channels (hCav3.2, encoded by the human CACNA1H gene and expressed in HEK293 cells), responsible for $I_{Ca,T}$, low threshold calcium current.
3. Cloned hHCN2 channels (encoded by the human HCN2 gene and expressed in CHO cells), responsible for I_f, hyperpolarization-activated cation current
4. Cloned hHCN4 channels (encoded by the human HCN4 gene and expressed in HEK cells), responsible for I_f, hyperpolarization-activated cation current.
5. Cloned hERG potassium channels (encoded by the KCNH2 gene and expressed in HEK293 cells).
6. Cloned hKir2.1 potassium channel (encoded by the human KCNJ2 gene and expressed in HEK293 cells), responsible for I_{K1}, inwardly rectifying potassium current.
7. Cloned hKir3.1/hKir3.4 potassium channels (expressed by the human KCNJ3 and KCNJ5 genes and co-expressed in HEK293 cells), responsible for I_{ACh}, inwardly rectifying potassium current.
8. Cloned Kir6.2/SUR2A potassium channels (expressed by the human KCNJ11 and SUR2A genes and co-expressed in HEK293 cells), responsible for the ATP sensitive current, I_{KATP}.
9. Cloned hKv1.5 potassium channels (encoded by the human KCNA5 gene and expressed in CHO cells), responsible for I_{Kur}, ultra-rapid delayed rectifier potassium current.
10. Cloned hKv4.3/KChiP2.2 potassium channels (encoded by the human KCND3 and KCNIP2 genes co-expressed in HEK293 cells).
11. Cloned hKvLQT1/hminK potassium channels (encoded by the human KCNQ1 and KCNE1 genes and co-expressed in CHO cells), responsible for I_{Ks}, slow delayed rectifier potassium current.
12. Cloned hNav1.5 sodium channels (SCN5A gene expressed in CHO cells).

Compounds were solubilized in physiological saline solution (HEPES buffer + 0.3% DMSO) at 30 µM. Experiments were conducted at room temperature using the QPatch HT®, an automatic parallel patch clamp system. MYK-461 and MYK-1078 were evaluated at a concentration of 30 µM and tested in at least 3 cells ($n \geq 3$). The duration of exposure to each test article concentration was at least 3 minutes. The positive control data confirmed the sensitivity of the test systems to ion channel inhibition.

Results

MYK-461 and MYK-1078 at 30 µM did not inhibit more than 13% of the current from all ion channels tested. MYK-461 and MYK-1078 inhibited hERG current by 7.8 and 4%, respectively.

In another study (Report #NC-19-0034), MYK-461 was tested at low concentrations of 1 and 10 µM. For all ion channels, inhibition at 10 µM was ≤ 6.9%, with the exceptions of hKir6.2/SUR2A (-50.5 ± 25.1%) and hKv1.5 (-7.6 ± 4.2%). hERG inhibition was 4.3 ± 1.9% at 10 µM.

4.3.6. Effect of MYK-461 and its metabolite on action potential parameters in isolated rabbit Purkinje fibers

This non-GLP study (Study #PFB-VA-50022, Report #NC-19-0036; Study #PFB-VA-50024, Report #NC-19-0037) was conducted at (b) (4) between July 31 and September 14, 2017. The objective of the study was to evaluate the cardiac cellular electrophysiological effects of MYK-461 (Report #NC-19-0036) and its metabolite MYK-1078 (Report #NC-19-0037) on the action potential parameters in isolated rabbit Purkinje fibers.

Methods

The arrhythmogenic potential of MYK-461 and MYK-1078 (at concentrations of 0.3, 3, 10, and 30 µM) was examined in isolated rabbit Purkinje fibers through a microelectrode technique. The fibers were superfused with an oxygenated physiological solution. After a 30-minute of stabilization, test articles were evaluated at increasing concentrations sequentially applied, every 30 minutes. For the control period and at each tested concentration, the fibers were stimulated at the basal rate of 1 pulse per second (1 Hz). The following parameters were measured: resting potential (RP in mV), amplitude (APA in mV), maximal rate of rise of action potential (Vmax in V/s), action potential duration at 50 and 90% of repolarization (APD50 and APD90 in ms). Both compounds were dissolved in DMSO.

Results

The acute effects of MYK-461 and MYK-1078 were evaluated at increasing concentrations in rabbit Purkinje fibers. MYK-461 at 30 µM induced a slight decrease in APD at 50% (-4.3 ± 1.0%) and 90% (-2.4%) of repolarization with no effects on the resting membrane potential or other AP parameters, suggests a mild inhibition of Na+ and/or Ca^{2+} channels. According to the sponsor, MYK-461 is devoid of electrophysiological effect in the Purkinje fiber preparation. Similarly, MYK-1078 from 0.3 to 30 µM, had no statistically significant effect either on the resting membrane potential or on the action potential parameters.

4.3.7. In vitro and in silico electrophysiological evaluation of MYK-461 and its metabolite

This non-GLP study (Report #NC-20-0061) was conducted at (b) (4) (report dated January 14, 2021). The objective of the study was to characterize the effects of MYK-461 and its metabolite MYK-1078 on human cardiac electrophysiology by examining effects on individual ion currents as well as on action potential morphology and duration both in vitro (HEK cells, and healthy and hypertrophied human primary ventricular myocytes) and in silico.

Methods

The acute and chronic effects of MYK-461 and MYK-1078 were evaluated on individual ion currents and on action potential morphology and duration in human embryonic kidney (HEK293) cells and human ventricular myocytes isolated from either healthy or diseased hypertrophied patients. Also, electrophysiological profile of MYK-461 was studied in silico via action potential modeling in line with the CiPA (comprehensive in vitro proarrhythmia assay) (IKr, ICaL, INaL, IKs, IK1 and I_{to}) initiative to optimize for arrhythmogenic assessments; models for both healthy and hypertrophic myocytes were used. In addition, the potential for MYK-461 to induce QT prolongation in human were evaluated via a proprietary QT fingerprinting in silico technique incorporating the effects on hERG, late-sodium (INaL), and L-type Ca2+ (Cav1.2, ICaL) currents.

The primary metabolites observed in rat plasma are M1 (MYK-2210) and M2 (MYK-1078), which are the oxidative metabolites formed in rat liver microsomes and rat hepatocytes. M2 is formed predominantly by CYP2C19. MYK-1078 constitutes <1% in human. The circulating level of MYK-1078 in human is <0.2 µM total at Cmax.

Results

a) Effects on Ion Currents: Acute (Table 24) or chronic (Table 25) exposure of MYK-461 either in ventricular myocytes or in expression system (HEK cells) was associated with a marked reduction (15 to 20% at concentrations as low as 3 µM) in Nav1.5 late/ INa (late) current amplitude (Fig. 37, left). A concentration-dependent (3 to 30 µM) reduction (-20% to -49% with an IC50 of 30.7 µM) in the current was observed in both matrices. Metabolite MYK-1078 was 3-fold more efficacious (IC50 9.4 µM vs 30.7 µM with MYK-461) than MYK-461 in blocking Nav1.5 (late)/INa (late) current (Table 25). At 30 µM, MYK-461 blocked Nav1.5 (late) 40.4 ± 1.2% whereas MYK-1078 blocked 79.3 ± 1.6%. Both MYK-461 (-14.5 ± 2.2 in hVM vs -22.8 ± 1.9% in hHVM) and MYK-1078 (-20.0 ± 2.1 in hVM vs -29.0 ± 2.8% in hHVM) reduced the INaL current, with a more robust effect observed in diseased cells (Table 24).

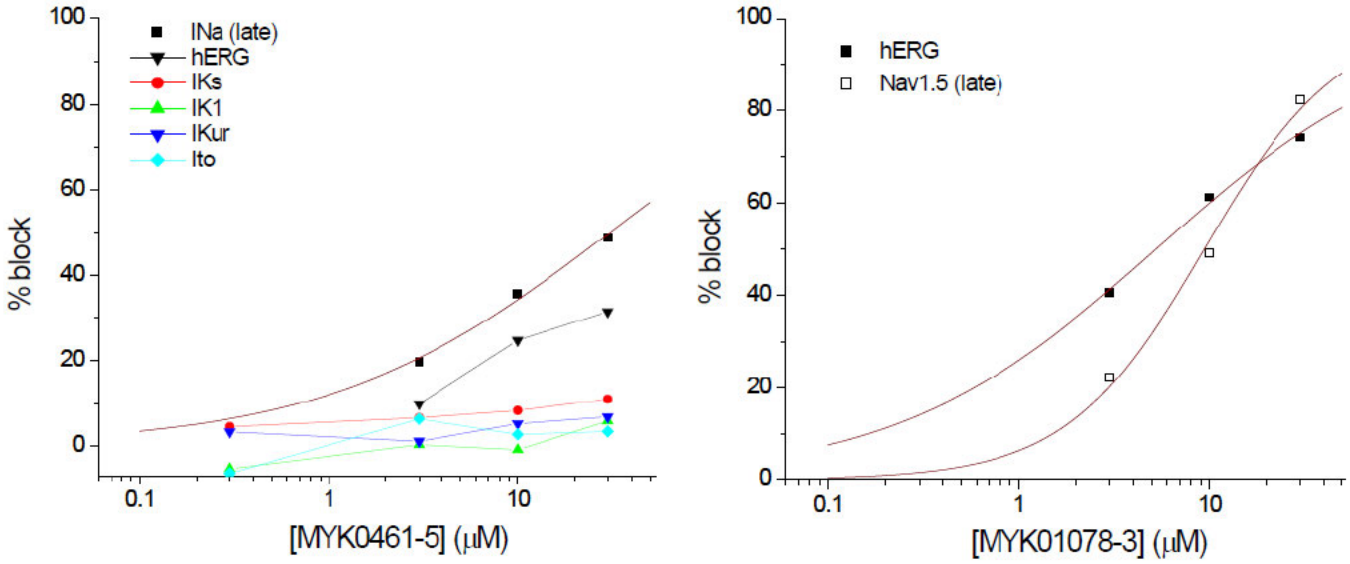


Figure 37. Chronic effects of MYK-461 (left) or MYK-1078 (right) on the indicated currents. Data was derived from Table 24 and 25. Symbols are mean ± SE

Table 24. Acute effects of MYK-461 and MYK-1078 in HEK in HEK cells and healthy and hypertrophied human primary ventricular myocytes (Reviewer's table)

Current	MYK-461 (concentration in µM)			MYK-1078 (concentration in µM)		
	3	10	30	3	10	30
Nav1.5 (late) Cells	-16.0 ± 1.3	-28.8 ± 1.6	-40.4 ± 1.2	-20.9 ± 1.5	-43.2 ± 3.0 (IC50 = 10.9 µM	-79.3 ± 1.6
late INa VM-healthy VM-hypert	-14.5 ± 2.2 -22.8 ± 1.9			-20.0 ± 2.1 -29.0 ± 2.8		
Cav1.2	Cells			VM		
	-2.5 ± 0.4	-6.9 ± 1.1	-14.7 ± 1.9	-2.0 ± 0.5	-4.9 ± 1.1	-10.3 ± 2.0
Kir6.2/SUR2A Cells	+0.03 ± 0.7	+2.7 ± 0.6	+4.3 ± 0.7	+0.2 ± 1.1	+4.1 ± 0.5	+4.3 ± 1.4
IKs, VM	0 ± 0	0 ± 0	-2.3 ± 0.8			
IK1, VM	0 ± 0	-3.4 ± 0.7	-4.9± 1.8			
IKur, VM	-2.2 ± 0.2	-4.3 ± 0.7	-8.3 ± 1.5			
Ito, VM	-2.6 ± 0.7	-5.9 ± 0.9	-12.4 ± 1.6			
Ikr (hERG) VM-healthy VM-hypert	-4.9 ± 0.9 -5.5 ± 1.7			-28.9 ± 2.4 -34.1 ± 1.5		

% change (mean ± SE) was calculated as the current amplitude after a steady state has been reached relative to the current amplitude before compound was applied (control). "-" means a decrease in current amplitude, "+" means an increase in current amplitude. Blank cells denote no studies were conducted. Cells: human embryonic kidney cells; VM: human primary ventricular myocytes

The acute effects of MYK-461 on IKr were slightly less (Table 24) than the chronic effects (Table 25) on the hERG current (4.9% reduction vs 9.9%, respectively at 3 µM) As with Nav1.5 late/ INa (late) current, MYK-1078 concertation-dependently blocked hERG current with an efficacy 4- to 5-fold higher than that of MYK-461 (Table 25) (Fig. 37, right). Comparable IKr responses were noted in both healthy and diseased human ventricular myocytes. Also, MYK-461 dose-dependently reduced the current amplitudes of Ik1, Ikur and Ito in ventricular myocytes under acute (Table 24) and chronic conditions (Table 25). In HEK cells, MYK-461 dose-dependently (3 to 30 µM) reduced the current amplitude of, Cav1.2 (-2.5 to -14.7%) (Table 24).

Table 25. Effects of chronic (48 h) application of MYK-461 and MYK-1078 on the indicated currents in healthy and hypertrophied human primary ventricular myocytes (Reviewer's table)

Current	MYK-461 (concentration in µM)				MYK-1078 (concentration in µM)		
	0.3	3	10	30	3	10	30
Late INa, VM		-19.7	-35.6*	-49.0* IC50 = 30.7 µM	-22.1*	-49.1* IC50 = 9.4 µM	-82.4*
IKs VM	-4.5	-6.6	-8.3	-11.0			
IK1, VM	+5.3	-0.3	+0.9	-5.8			
IKur, VM	-3.3	-1.1	-5.2	-6.8			
Ito, VM	+6.4	-6.3	-2.7	-3.4			
hERG, Cells		-9.9	-24.7*	-31.5*	-40.6*	-61.3* IC50 = 5.3 µM	-74.2*

b) Effects on Action Potential: In healthy human ventricular myocytes, MYK-461 concentration-dependently (0.3 to 30 µM) shortened the action potential duration (APD90, -12 to -25%) (Fig. 38, Left), while the metabolite MYK-1078 concentration-dependently prolonged the APD (APD90, +3.6 to +13.4%) (Fig. 39, Left). This differential effect between the parent compound and the metabolite is probably the result of a greater blockade of Ikr than INa late by the metabolite. Neither compound was associated with early afterdepolarizations (EADs, that is triggering extra beats) or substantive triangulation, biomarkers for arrhythmogenesis. The APD effects were similar in myocytes isolated from hypertrophied hearts (Fig. 38, Right and 39, Right). The positive control, dofetilide, prolonged APD and that was reduced with the addition of MYK-461.

c) In Silico: The in-silico model predicted a small increase in APD under healthy conditions (+9.8% at 30 µM) and less of an increase under hypertrophied conditions (+3% at 30 µM). Under no conditions are there any indications of EADs. When INa and ICa are omitted from the model and there is just outward current block, there is a much larger increase in APD and under some conditions EADs. Incorporating the effects on hERG, late-sodium (INaL), and L-type Ca2+ (Cav1.2, ICaL) currents, QT fingerprinting

in silico technique predicted a lack of QT prolongation or a QTc shortening for MYK-461 and QTc prolongation for the metabolite MYK-1078.

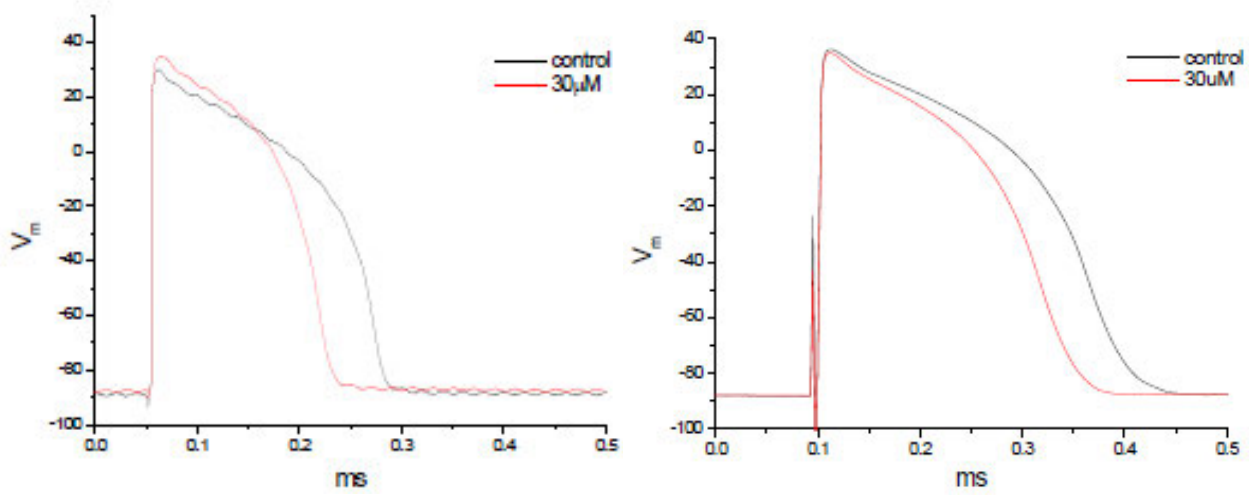


Figure 38. Effects of MYK-461 (at 30 μM) on action potential morphology recorded from a healthy human ventricular myocyte (left) or hypertrophied ventricular myocyte (right).

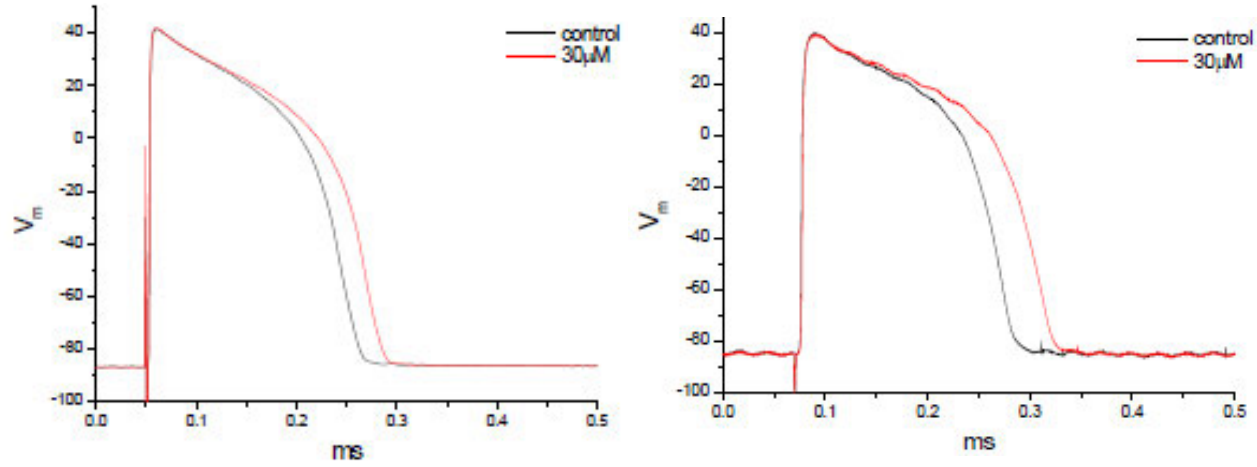


Figure 39. Effects of MYK-1078 (at 30 μM) on action potential morphology recorded from a healthy human ventricular myocyte (left) or hypertrophied ventricular myocyte (right).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 Absorption

5.1.1 Pharmacokinetics of MYK-461 following a single intravenous or oral administration across species

Several non-GLP studies were conducted in a few animal species to assess the pharmacokinetic properties and the bioavailability of MYK-461 after a single intravenous or oral administration in male mice, male Wistar Han rats, beagle dogs and monkeys.

Methods

MYK-461 was administered intravenously as a single bolus dose (formulated in DMA:PEG400:30% HPβCD/normal saline (5:25:70)) in mice, rats, dogs and monkeys (Table 26). Same solution was administered orally by gavage for mice and rats. For dogs, a 0.5% methylcellulose suspension formulation was used. For Cynomolgus monkeys, an OraSweet/Oraplus (50:50) suspension formulation was utilized (Table 27).

Results

No mortality or treatment-related clinical signs were observed in any of the animals receiving i.v. or oral doses of MYK-461.

Following bolus i.v. administration of MYK-461, concentration-time profiles demonstrated rapid distribution phase followed by monoexponential decay. The elimination half-life ($t_{1/2}$) was longer for dogs (130 h) than for monkeys (44.5 h). It was relatively short for rats (11 h) and mice (7 h). Test substance was cleared at a low rate, 2% of liver blood flow in the dog and ranged from 7 to 10% of liver blood flow in the mouse, rat and monkey. The mean volume of distribution at steady state was high in monkeys (10.6 L/kg), moderate in dogs (7 L/kg), and low in rats (5 L/kg) and mice (3.8 L/kg) (Table 26).

Following oral administration, MYK-461 plasma concentration reached peak between 0.3 and 0.7 hr. The absorption profile is consistent with MYK-461's classification of Class II in the biopharmaceutical classification system: low solubility (0.02 mg/mL) and high permeability. Oral bioavailability of MYK-461 was high in all species studied. The mean half-life was shorter in mice (4 to 6 hr) and rats (8 hr) than in monkeys (43 h) and dogs (161 h) (Table 27).

Table 26. Mean PK parameters of MYK-461 after a single intravenous bolus dose across species

Study #	NC-14-0015	NC-14-0018	NC-14-0024	NC-14-0028
Test Article	MYK-461	MYK-461	MYK-461	MYK-461
Batch	MYK-461 lot 2	MYK-461 lot 2	MYK-461 lot 2	MYK-461 lot 6
Species	C57/BL6 Mouse	Sprague Dawley Rat	Beagle Dog	Cynomolgus Monkey
No. of Animals	27M	3M	3M	3M
Feeding Condition	Fasted	Fasted	Non-fasted	Non-fasted
Vehicle/Formulation	DMA:PEG400:30% normal saline (5:10:85)	DMA:PEG400:30% HPβCD (5:25:70)	DMA:PEG400:30% normal saline (5:10:85)	DMA:PEG400:30% normal saline (5:10:85)
Analyte	MYK-461	MYK-461	MYK-461	MYK-461
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Dose (mg/kg)	**1**	**1**	**0.25**	**0.25**
Mean (%CV) PK Parameters				
Cmax (ng/mL)	853	869 (101)	219 (52.2)	70.6 (24.0)
AUC(0-∞) (ng*h/mL)	2180	2880 (9.1)	4210 (30.2)[a]	1520 (32.5)
AUC(0-∞)/dose	2180	2880	16840[a]	6080
CL (mL/min/kg)	7.65	5.82 (8.70)	0.584 (20.1)	2.98 (36.0)
%Liver Blood Flow	8.5	10.6	1.9	6.8
V_{SS} (L/kg)	3.81	5.01 (14.5)	7.01 (38.2)	10.6 (20.2)
Fold over Total Body Water	5.3	7.5	11.6	15.3
$t_{1/2}$ (h)	7.02	11.2 (9.30)	130 (15.7)	44.5 (17.5)

a: Represented by $AUC_{0\text{-}168h}$ and $AUC_{0\text{-}168h}$/dose.
Mean (%CV) values were reported except for mice, in which a single value from a composite PK profile was reported based on sparse sampling across multiple animals.
Additional information: The concentration-time profiles of mavacamten can be characterized by bi-exponential decay with a rapid distribution phase and a slow elimination phase. The dose-normalized AUC was similar across species except for dog where the exposures were higher. Accordingly, dogs had the lowest in vivo clearance (<2% of liver blood flow vs. 7 to 10% for other species). MYK-461 exhibited a high steady-state volume of distribution across species ranging from 5 to 15-fold greater than total body water.

Table 27. Mean PK parameters of MYK-461 after a single oral gavage administration across species

Study #	NC-14-0015	NC-14-0016	NC-14-0018	NC-14-0024	NC-14-0028
Test Article	MYK-461	MYK-461	MYK-461	MYK-461	MYK-461
Batch	MYK-461 lot 2	MYK-461 lot 6	MYK-461 lot 2	MYK-461 lot 2	MYK-461 lot 6
Species	C57/BL6 Mouse	129SvEv Mouse	Sprague Dawley Rat	Beagle Dog	Cynomolgus Monkey
No. of Animals	24M	30M	3M	3M	3M
Feeding Condition	Fasted	Non-fasted	Fasted	Fasted	Non-fasted
Vehicle/Formulation	DMA:PEG400:30% 0.9% NaCl (5:10:85)	DMA:PEG400:30% HPβCD (5:25:70)	DMA:PEG400:30% HPβCD (5:25:70)	0.5% methylcellulose	Sweet/OraPlus (50:50)
Analyte	MYK-461	MYK-461	MYK-461	MYK-461	MYK-461
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Dose (mg/kg)	**1**	**2.5**	**2**	**0.5**	**0.5**
Mean (%CV) PK Parameters					
Tmax (h)	0.5	0.25	0.7 (43.3)	0.3 (0.0)	0.7 (43.3)
Cmax (ng/mL)	564	1120	522 (17.9)	186 (10.7)	63.0 (37.2)
AUC(0-∞) (ng*h/mL)	3170	16100	4310 (25.5)	7330 (10.0)[a]	1410 (32.9)
AUC(0-∞)/dose	3170	6440	2160 (25.5)	14700 (10.3)[a]	2820 (32.9)
t1/2 (h)	4.18	6.40	8.20 (12.5)	161 (46.0)	42.7 (14.3)
% Bioavailability[b]	145	NC	74.8 (25.6)	87.1 (10.4)	46.5 (32.9)

a Presented as $AUC_{0\text{-}168h}$ and $AUC_{0\text{-}168h}$/dose due to extensive extrapolation of terminal phase.
b Calculated using IV exposures presented in Table 26.
NC = not calculated
Mean (%CV) values were reported except for mice, in which a single value from a composite PK profile was reported based on sparse sampling across multiple animals.

The effect of ad libitum food on the absorption of mavacamten was investigated in the rat (NC-14-0019) and the dog (NC-14-0026).

The presence of food did not markedly affect half-life and Tmax of MYK-461. The fed/fasted ratios of mean Cmax and $AUC_{0\text{-}24}$ were 0.51 and 0.76, respectively, with similar intersubject variability across feeding regimens. These data suggest that ad libitum food intake resulted in modestly reduced AUC exposures (24% decrease) in the rat. In the dog, the presence of food did not affect half-life and Tmax. The fed/fasted ratios of mean Cmax (178/176 ng/ml) and $AUC_{0\text{-}24}$ (7100/7040 ng*h/ml) were both 1.01. The t1/2 was slightly higher in fed (136 h) than in the fasted (104 h) state.

5.2. Distribution

5.2.1 In vitro plasma protein-binding and blood to plasma ratio

This non-GLP study (NC-17-0016) was conducted at MyoKardia (report dated 11/22/2019). The objectives of the study were to 1) determine the extent of MYK-461 binding to proteins in the mouse, rat, monkey, dog and human plasma; 2) determine the blood to plasma ratio of MYK-461 across species.

The extent of protein binding [^{14}C] MYK-461 was determined at concentrations ranging from 0.2 µM to 10 µM in the mouse, rat, monkey dog and human plasma at 37°C.

MYK-461 was modestly bound to protein in all of the species studied, and it ranged from 83.6% bound in the mouse to 96.9% bound in the monkey. The binding of MYK-461 did not depend on the concentration within species (Table 28).

Table 28. Summary of MYK-461 binding to plasma proteins of the mouse, rat, monkey, dog and human plasma

Species	% Bound			Average over the concentration range	
	0.2 µM	1 µM	10 µM	Average	SD
Mouse	83.6	84.2	84.0	83.9	0.3
Rat	89.4	89.1	88.5	89.0	0.5
Dog	92.6	91.9	88.8	91.1	2.0
Monkey	96.9	96.5	91.9	95.1	2.8
Human	92.9	93.3	93.1	93.1	0.2

The blood-to-plasma partitioning of MYK-461 was determined in vitro across species. Blood-to-plasma ratios measured in the mouse, rat, dog, cynomolgus monkey ad human ranged between 0.72 ± 0.10 (mouse) and 0.82 ± 0.09 (monkey). The blood-to-plasma ratio determined from human blood was 0.79 ± 0.12. The data suggest that MYK-461 is primarily partitioned into the plasma and therefore, plasma is a representative of blood pharmacokinetics and an appropriate matrix for PK evaluation of MYK-461.

4.2.2 Tissue distribution studies in rats

Two non-GLP studies (NC14-0023 and NC-19-0007) were conducted at Myokardia. The objectives of these studies were to 1) determine the radioactivity in tissues after administration of [14C]MYK-461 by Quantitative Whole Body Autoradiography (QWBA) (Report #NC-0007) and 2) distribution of MYK-461 in selected tissues and striated muscle after single and repeated oral doses.

A single oral dose of 1 mg MYK-461/kg (formulated in 0.5% methyl cellulose in water) was administered by gavage to 3 male Sprague-Dawley rats. Another group of 3 male S-D rats received the same dose but for 7 consecutive days. Blood was collected on 0.25, 0.5, 1, 2, 4, 6, and 24 h on day 1 or day 7. Tissues were harvested at 24 h post the last dose (day 1 or day 7) (Report #NC14-0023).

For autoradiography study (Report #NC-19-0007), a single oral dose of 1 mg [14C]MYK-461/kg (formulated in DMA:PEG400:30%HPβCD) was administered by gavage to 9 male Long Evans (pigmented) rats (one each sacrificed at 0.5, 1, 4, 8, 24, 72, 168, 336 and 720 h post dose) and 5 male S-D (non-pigmented) rats (one rat sacrificed at 1, 4, 24, 48 and 168 hours post dose). Animals were prepared for QWBA at the time indicated above. Sections were collected at five levels of interest in the sagittal plane with all major tissues, organs, and biological fluids represented. The autoradiographic standard image data were sampled to create a calibrated standard curve and from which tissue concentrations were interpolated as nanocuries/g and then converted to ng equivalents/g on the basis of the test article specific activity.

Results

Following oral administration of nonradioactive MYK-461, high concentrations of test substance were noted on day 1 in the skeletal (soleus) muscle followed by heart. Extensor digitorum longus and heart to plasma had tissue to plasma ratios of MYK-461 of approximately 10. Soleus had a higher MYK-461 tissue to plasma ratio at 20. On the other hand, smooth muscle-to-plasma ratio, represented by the esophagus, and organs of filtration (liver and kidney) was similar, ~3:1. MYK-461 distribution into clearance organs, liver and kidney, was moderate with tissue to plasma ratios of approximately 3. It was not detected in the brain (Table 29).

Table 29. Tissue distribution of MYK-461 in the rat following a single or 7-day oral dose in steady state

		Plasma	Liver	Spleen	Lung	Esophagus	Soleus	EDL	Heart	Kidney	Brain
MYK-461 concentration (ng/mL)											
Day 1	Mean	48.9	163	22.2	86.6	181	824	503	476	139	1.69
	SD	59.6	190	17.5	110	266	702	584	465	165	2.93
	%CV	122	117	79.0	127	147	85.2	116	97.6	119	173
Day 7	Mean	40.0	123	15.3	52.6	152	743	365	375	101	BLQ
	SD	29.7	103	15.9	44.8	122	526	257	292	96.0	NA
	%CV	74.3	84.2	103.8	85.3	80.0	70.8	70.5	77.8	95.2	NA
Tissue-to-plasma ratio											
Day 1	Mean	NA	3.40	0.355	1.59	3.08	23.5	10.2	11.6	2.86	NA
	SD	NA	0.344	0.0845	0.251	1.52	9.54	2.28	4.14	0.285	NA
	%CV	NA	10.1	23.8	15.8	49.2	40.6	22.4	35.5	10.0	NA
Day 7	Mean	NA	2.91	0.255	1.25	3.70	18.9	9.34	9.14	2.28	NA
	SD	NA	0.373	0.147	0.138	0.213	0.665	0.718	0.909	0.503	NA
	%CV	NA	12.8	57.7	11.1	5.8	3.5	7.7	9.9	22.1	NA

NA = not applicable; BQL = below quantification limit

EDL = extensor digitorum longus

Following oral administration of 1 mg [14C]MYK-461 to LE pigmented and S-D non-pigmented rats, radioactivity was rapidly absorbed (noted as early as 0.5 h, the first time point) and extensively distributed in tissues and organs. In pigmented rats, peak radioactivity was noted between 168 and 336 h post dose. Highest concentrations were observed in the heart, diaphragm, liver, salivary gland, skeletal muscle, and esophagus. The radioactivity distributed preferentially to the heart, diaphragm, esophagus, stomach mucosa, small intestine, secretory glands, and select areas of skeletal muscle proximal to cortical bone. Low radioactivity concentrations were documented in central nervous system tissues (cerebellum, cerebrum, medulla, and spinal cord). Low levels of [14C]MYK-461-derived radioactivity were selectively associated with melanin-containing tissues of the eye suggesting MYK-461 has a significant affinity for melanin in the skin (Table 30). For the non-pigmented rat, distribution trends were generally comparable to those seen in pigmented male rat.

Table 30. Tissue:plasma concentration ratios at specified times after a single oral administration of 1 mg/kg [14C]MYK-461 to male Long Evans rat

	Tissue:Plasma Concentration Ratio Animal Number (Euthanasia Time)								
Tissue	B40677 (0.5 h)	B40678 (1 h)	B40679 (4 h)	B40680 (8 h)	B40681 (24 h)	B40682 (72 h)	B40683 (168 h)	B40684 (336 h)	B40685 (720 h)
Adrenal gland(s)	1.28	1.28	1.51	1.38	2.16	4.41	6.84	6.14	NA
Arterial wall	1.13	1.45	1.25	1.06	2.29	6.71	11.2	28.2	NA
Blood	0.620	0.640	0.771	0.679	0.835	NA	NA	NA	NA
Bone	0.0481	0.0457	0.0783	0.0699	NA	NA	NA	NA	NA
Bone marrow	0.531	0.532	0.542	0.632	0.816	1.35	2.01	NA	NA
Brain cerebellum	0.0311	0.0612	0.125	0.197	0.669	4.36	6.31	6.42	NA
Brain cerebrum	0.0354	0.0619	0.137	0.201	0.738	5.57	7.79	8.46	NA
Brain medulla	0.0356	0.0791	0.134	0.201	0.881	6.61	9.32	10.7	NA
Brain olfactory lobe	0.0315	0.0442	0.0663	0.150	0.788	5.34	10.4	9.53	NA
Bulbo-urethral gland	0.776	0.817	0.873	0.934	1.38	3.18	4.33	NA	NA
Cecum	0.558	0.853	1.01	1.11	1.12	8.54	11.3	13.9	NA
Diaphragm	4.53	9.03	9.82	9.78	15.0	65.0	74.5	95.3	NA
Epididymis	0.261	0.565	0.587	0.254	0.496	NA	NA	NA	NA
Esophagus	2.20	4.60	4.85	5.08	5.04	15.3	21.6	15.8	NA
Exorbital lacrimal gland	0.812	0.788	0.970	1.05	1.63	2.48	4.07	NA	NA
Eye lens	0.0128	0.0254	0.0555	0.0496	0.198	NA	NA	NA	NA
Eye uveal tract	0.675	0.622	0.928	1.10	2.27	16.7	27.6	80.9	NA
Eye(s)	0.109	0.165	0.198	0.303	0.504	4.52	4.60	13.0	NA
Fat (abdominal)	0.356	0.285	0.330	0.318	0.390	NA	NA	NA	NA
Fat (brown)	0.699	0.888	1.37	1.46	2.88	6.19	18.1	18.2	NA
Harderian gland	1.35	1.56	1.60	1.66	2.73	1.88	7.19	9.08	NA
Intra-orbital lacrimal gland	0.806	0.863	0.873	0.919	1.36	2.10	3.53	NA	NA
Kidney cortex	2.14	2.62	4.08	4.03	9.37	49.4	73.8	182	NA
Kidney medulla	1.44	2.05	2.41	2.63	6.75	37.8	47.9	67.5	NA

	Tissue:Plasma Concentration Ratio Animal Number (Euthanasia Time)								
Tissue	B40677 (0.5 h)	B40678 (1 h)	B40679 (4 h)	B40680 (8 h)	B40681 (24 h)	B40682 (72 h)	B40683 (168 h)	B40684 (336 h)	B40685 (720 h)
Kidney(s)	2.02	2.41	3.47	3.48	8.87	47.1	84.0	169	NA
Large intestine	0.525	0.590	0.741	1.10	5.28	8.68	8.86	7.78	NA
Liver	4.59	3.10	4.04	3.49	3.31	7.80	10.9	10.4	NA
Lung(s)	0.650	0.601	0.651	0.667	1.09	1.74	NA	NA	NA
Lymph node(s)	0.505	0.554	0.639	0.566	0.643	2.04	NA	NA	NA
Muscle	1.42	3.53	7.95	7.94	9.46	37.4	45.6	37.3	NA
Myocardium	8.67	13.6	16.1	14.9	29.4	101	151	189	NA
Nasal turbinates	0.206	0.251	0.491	0.525	1.43	5.13	4.94	6.85	NA
Pancreas	0.754	0.676	0.723	0.838	0.825	NA	NA	NA	NA
Pituitary gland	0.727	0.701	0.669	0.729	0.790	1.61	NA	NA	NA
Preputial gland	0.509	0.655	0.558	0.607	1.13	7.22	NA	NA	NA
Prostate gland	0.475	0.687	0.663	0.704	0.797	1.35	NA	NA	NA
Salivary gland(s)	2.30	5.18	10.2	12.6	10.5	22.2	24.3	26.1	NA
Seminal vesicle(s)	0.267	0.417	0.570	0.371	0.487	NA	NA	NA	NA
Skin (nonpigmented)	0.366	0.514	0.529	0.455	0.649	1.54	NA	NA	NA
Skin (pigmented)	0.453	0.579	0.633	0.535	0.814	4.15	2.23	NA	NA
Small intestine	1.48	2.19	3.29	3.26	3.01	3.46	NA	NA	NA
Spinal cord	0.0442	0.0813	0.0614	0.286	0.686	9.74	9.85	7.01	NA
Spleen	0.558	0.511	0.537	0.504	0.662	NA	NA	NA	NA
Stomach	0.539	0.935	0.855	1.10	1.96	NA	NA	NA	NA
Stomach mucosa	1.32	1.55	2.36	2.58	5.28	25.3	25.2	32.5	NA
Stomach wall	0.622	0.493	0.581	0.523	0.846	NA	NA	NA	NA
Testis(es)	0.0947	0.214	0.259	0.235	0.297	NA	NA	NA	NA
Thymus	0.434	0.446	0.387	0.390	0.394	NA	NA	NA	NA

	Tissue:Plasma Concentration Ratio Animal Number (Euthansia Time)								
Tissue	B40677 (0.5 h)	B40678 (1 h)	B40679 (4 h)	B40680 (8 h)	B40681 (24 h)	B40682 (72 h)	B40683 (168 h)	B40684 (336 h)	B40685 (720 h)
Thyroid	0.853	1.46	2.25	1.77	2.51	6.98	5.67	8.67	NA
Urinary bladder	0.749	0.824	0.614	0.687	2.07	NA	NA	NA	NA

h Hours
NA Not applicable

4.2.3 Tissue distribution studies in dogs

This non-GLP study ((b) (4)13-1168) was conducted at MyoKardia, (b) (4). The objective of the study was to determine the distribution of MYK-461 in cardiac and skeletal muscle after a single oral dose in dogs.

Beagle dogs (n=1/dose/sex) were randomized to receive one of the three escalating single doses of MYK-461 (1.5, 4.5 or 7.5 mg/kg). Dose formulations were prepared in 0.5% methylcellulose in reverse osmosis water and administered orally via gavage at a constant dose volume of 10 ml/kg. Dogs were sacrificed 7 days post dose administration; heart and skeletal muscle were collected.

Results

Following oral administration of MYK-461, high concentrations of test substance were noted on post dose day 7 in both skeletal muscle and the heart. The findings reiterate the long half-life of MYK-461 in dogs. Tissue-to-plasma ratios ranged between 8.6:1 and 30.5:1 (Table 31).

Table 31. Tissue distribution, and heart and skeletal to plasma ratios of MYK-461 in the dog following a single oral dose at steady state

Dose (mg/kg)	1.5	4.5	7	
Sample collection time post dose	7 days	7 days	7 days	
Sex	Female	Female	Female	Male
MYK-461 concentrations (ng/mL)				
Plasma	35.8	56.1	53.7	88.9
FDL	306	1080	1100	2710
EDL	378	859	963	1850
LV	450	992	1260	1850
MYK-461 tissue:plasma ratio				
FDL:Plasma	8.55	19.3	20.5	30.5
EDL:Plasma	10.6	15.3	17.9	20.8
LV:Plasma	12.6	17.7	23.5	20.8

FDL = flexor digitorum longus; EDL = extensor digitorum longus; LV = left ventricle

4.3. Metabolism

4.3.1 *In* vitro CYP450 enzyme inhibition of MYK-461 in human microsomes

This non-GLP study (NC-17-0018, report dated December 18, 2020) was conducted at MyoKardia. The objective of the study was to evaluate the potential of specific CYP450 enzymes in the metabolism of MYK-461 using human recombinant CYP enzymes and human liver microsomes.

Methods

Studies were conducted with human recombinant CYP enzymes and human liver microsomes with chemical selective inhibitors to identify the CYP enzymes involved in the metabolism of MYK-461. For the cDNA expressed enzyme reactions, 1 µM MYK-461 was incubated with the enzyme and NADPH for 60 min. The amount of test article remaining relative to the amount at time 0 was noted. In the second study, pooled human liver microsomes were incubated with MYK-461 (10 µM) for 60 min with and without NADPH. Specific marker substrates were used to evaluate the selected CYPs such as 1A2 (α-naphthoflavone), 2B6 (thioTEPA), 2C8 (montelukast), 2C9 (sulfaphenazole), 2C19 ([+]-N-3-benzylnirvanol), 2D6 (quinidine), 2E1 (tranylcypromine), and 3A4/5 (ketoconazole and azamulin).

Results

Studies with human recombinant CYP enzymes showed MYK-461 was metabolized primarily by 2C19 (71.4% reduction) followed by 3A4 (43.6% reduction), and 3A5 (57.2% reduction). Also, some metabolism (10-20% reduction) was detected with recombinant CYP enzymes 1A2, 2B6, 2C8, 2C9, 2D6, and 2J2 (Fig. 40).

Studies with individual recombinant CYP enzymes showed that metabolite M1 (MYK-2210) was formed to a great extent with CYP2C9 and 2C8. Additionally, metabolite M2 (MYK-1078) was formed predominantly with CYP2C19. Besides, metabolite M6 (MYK-2241) was formed with CYPs 3A5 and 3A4; metabolite M7 was formed to the highest extent in incubations with CYPs 2C9 but also by 2D6, 2C19, 3A5; metabolite M8 (MYK-1065) was formed predominantly by CYPs 3A5 but also by 3A4; and finally, metabolite M9 was formed mostly in incubations with CYP 2C19 (Table 32).

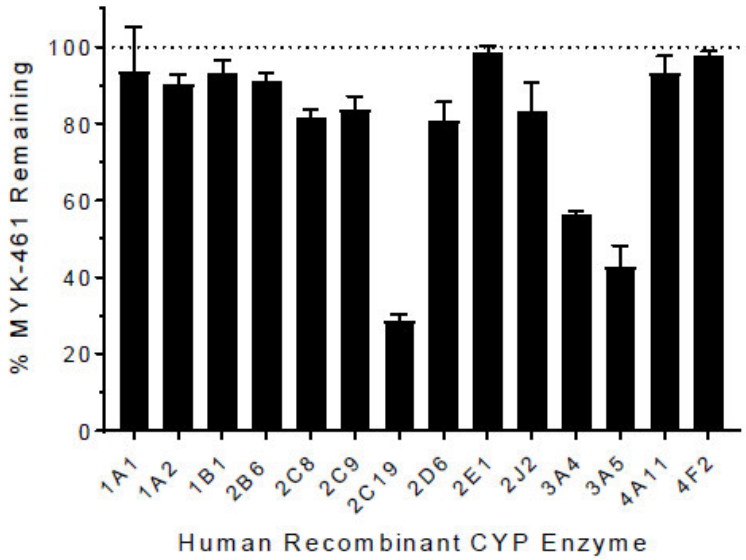


Figure 40. Percent MYK-461 metabolized by select cDNA expressed enzymes

Table 32. Relative amounts of MYK-461 metabolite formed in incubations with human recombinant CYP enzymes

rCYP	% of Maximum Formed for each Metabolite Across rCYP[a]					
	M1	M7	M8	M2	M6	M9
1A2	13.3	21.3	ND	0.139	0.64	ND
2B6	ND	ND	ND	0.003	1.66	ND
2C8	63.9	10.8	ND	2.10	1.97	4.23
2C9	**100**	**100**	ND	0.22	4.20	50.7
2C19	20.7	66.6	ND	**100**	ND	**100**
2D6	25.5	72.6	ND	12.4	0.86	23.7
2E1	ND	ND	ND	0.02	0.74	ND
3A4	9.50	25.3	22.8	3.62	**100**	15.2
3A5	15.0	49.1	**100**	12.3	76.4	29.0

ND, not detected.
Note: MYK-461 (30 µM) incubation with rCYP enzymes.
[a] Calculated by dividing the metabolite peak area ratio by the peak area ratio of the metabolite having the largest peak area ratio and then multiplying by 100

CYP reaction phenotyping was assessed in inhibition studies conducted with human liver microsomes. The results demonstrated the role of CYPs 2C19, 3A4/5, and 2C9 in the metabolism of MYK-461. The metabolism of MYK-461 in liver microsomes leading to the formation of hydroxylated metabolite M1 and metabolite M2 was inhibited primarily by CYP2C9 (sulfaphenazole) and CYP2C19 ((+)-N-3-benzylnirvanol),

respectively. The contribution of CYPs 2C19, 3A4/5 and 2C9 in the metabolism of MYK-461 was 74.3, 18.0, and 7.55%, respectively. Metabolism of MYK-461 by CYP2J2 was negligible (Table 33).

Table 33. Contribution of select CYP enzymes to the hepatic metabolism of MYK-461

CYP Enzyme	Abundance in pmol CYP/mg HLM protein (% of Total Abundance in Native HLM)	Metabolite Formation Scaling (pmol/min/mg HLM protein)							Contribution Based on f_m (%)
		M1 MYK-2210	M2 MYK-1078	M5 MYK-1096	M6 MYK-2241	M8 MYK-1065	M9 MYK-2364	All Six Metabolites	
CYP 3A4	137 (31%)	38.8	86.2	36.9	121	44.9	31.5	293	18.0
CYP 2C9	73 (16%)	81.1	13.8	0.00	21.7	13.1	21.6	134	7.55
CYP 2C19	14 (3.2%)	8.59	1360	1.33	2.15	112	3.21	1352	74.3
CYP2J2	1.2 (0.27%)	0.35	1.31	0.12	0.17	2.08	0.18	3.82	0.21
Total	443.6	129	1460	38.4	145	172	56.5	1783	100

HLM = human liver microsomes
f_m = fraction metabolized

4.3.2 *In* vitro CYP450 enzyme inhibition of MYK-461 in human microsomes

This non-GLP study (NC-17-0017, report dated November 17, 2019) was conducted at MyoKardia. The objective of the study was to determine the metabolic profile of [^{14}C]MYK-461 in vitro with liver microsomes and hepatocytes across species. Also, structural elucidation of primary metabolites was performed.

Methods

In the first study, [^{14}C]MYK-461 was incubated with liver microsomes from mice, rats, dogs, monkeys, and human; and in the second study, it was incubated with hepatocytes from rats, dogs, monkeys, and human. [^{14}C]MYK-461 metabolites were characterized across species by LC-MS/MS detection.

Results

In liver microsomes, [^{14}C]MYK-461 produced low levels of metabolites in all species studied. A total of thirteen [^{14}C]MYK-461 metabolites were detected, identified, and characterized across species. Predominant metabolites identified across species were M1, M2, M6 and M12 (Table 34). Hydroxylation of the phenyl or isopropyl moieties produced metabolites M1 and M2, respectively. Oxidative N-dealkylation of test

substance produced M6. All of these metabolites were NADPH-dependent indicating that they were formed by metabolism. However, M6 was seen at low levels in a control incubation that was missing NADPH suggesting its production is not entirely driven by NADPH-dependent processes.

In hepatocytes, the biotransformation of MYK-461 produced additional four metabolites besides M1, M2 and M6 previously observed in liver microsomes. The microsomal metabolite M12 was not detected in hepatocytes from any species. Glucuronidation of M1 produced the *O*-glucuronide, M4, in the rat but not in the dog, monkey or human. Additionally, the rat-specific metabolite, M10, was characterized as a putative conjugate of the pyrimidinedione portion of MYK-461. Two unique monkey metabolites were characterized: hydroxylation of the phenyl moiety and hydrolysis of the pyrimidinedione portion of the molecule produced M11, while hydroxylation of the pyrimidinedione portion of the molecule followed by glucuronidation produced M13 (Table 35).

Table 34. Relative amounts of [14C]MYK-461 and identified metabolites as a percentage of total radioactivity following incubation in animal and human liver microsomes

Component	Peak Areas (%)				
	Mouse	Rat	Dog	Monkey	Human
MYK-461	92.6	93.0	97.7	94.5	96.1
M1	1.44	3.46	0.770	0.530	0.850
M2	0.540	0.470	0.320	2.10	0.820
M6	1.88	2.13	0.640	1.92	1.35
M12	3.01	0.970	0.530	0.930	0.910
Uncharacterized	0.510	N.D	N.D	N.D	N.D

N.D., not detected

Table 35. Relative amounts of [14C]MYK-461 and identified metabolites as a percentage of total radioactivity following incubation in animal and human hepatocytes

Component	Peak Areas (%)			
	Rat	Dog	Monkey	Human
MYK-461	80.4	99.6	89.0	96.9
M1	2.84	N.D	1.76	0.780
M2	1.15	N.D	2.71	0.940
M4	9.01	N.D	N.D	N.D
M6	4.41	0.380	3.21	0.770
M10	0.790	N.D	N.D	N.D
M11	N.D	N.D	1.30	N.D
M13	N.D	N.D	2.04	N.D
Uncharacterized	1.42	0.00	0.00	0.630

N.D., not detected

4.3.3 *In* vitro evaluation of MYK-461 as an inhibitor of CYP450

This non-GLP study (#NC-19-0019, report dated November 30, 2016)) was conducted at (b) (4) The objective of the study was to evaluate the potential of MYK-461 to inhibit CYP450 enzymes in human liver microsomes.

Methods

Pooled liver microsomes from 50 human (mixed gender) donors were obtained from a commercial source. A summary of the CYP isoform and its substrate used in the study is summarized in Table 36.

Each isoform-specific CYP450 probe substrate was incubated in the presence of MYK-461 (up to 200 µM). IC_{50} values for the inhibition of 8 CYP isoforms (CYP1A2, CYP2B6, CYP2C9, CYP2C8, CYP2C19, CYP2D6, CYP3A4/5) by MYK-461 were determined.

Results

MYK-461 inhibited CYP2C9 and CYP2C19 with IC50 values of 82.2 µM and 89.7 µM, respectively. Additional studies were conducted with these two enzymes, that revealed apparent inhibition constants (Ki) of 59.5 µM and 46.2 µM for CYP2C9 and CYP2C19, respectively. On the other hand, MYK-461 did not inhibit CYP1A2, CYP2B6, CYP2C8, and CYP2D6 up to the maximum concentration tested. Furthermore, weak inhibition of CYP2C9, CYP2C19, and CYP3A4/5 but only at concentrations outside the therapeutic range was noted. Based on these findings, the sponsor concludes that a low potential for drug-drug interaction via competitive CYP inhibition exists for MYK-461 (Table 36).

Table 36. Inhibition of human liver CYPs by MYK-461

CYP Enzyme	Probe Substrate	Mavacamten IC_{50} (µM)
CYP1A2	phenacetin	no inhibition
CYP2B6	bupropion	no inhibition
CYP2C8	amodiaquine	no inhibition
CYP2C9	diclofenac	82.2
CYP2C19	S-mephenytoin	89.7
CYP2D6	dextromethorphan	no inhibition
CYP3A4/5	midazolam	activation
CYP3A4/5	testosterone	175

4.3.4 *In* vitro evaluation of MYK-461 as an inductor of CYP450

This non-GLP study (NC-19-0006, dated July 30, 2014; NC-19-0012, dated June 19, 2017) was conducted at (b) (4) The objective of the study was to determine the induction potential of CYP450 enzymes by MYK-461 in cultured human hepatocytes. Induction was measured by mRNA expression and catalytic activity assays selective for CYP1A2, CYP2B6, CYP3A4, CYP2C8, CYP2C9, and CYP2C19. Additionally, toxic potential of MYK-461 to human hepatocytes was conducted.

Methods

Cryopreserved human hepatocytes (4 donors of mixed gender) were obtained commercially. MYK-461 was incubated at 37ºC with hepatocytes at concentrations of 0.05, 0.15, 0.5, 1.5, 5, and 15 µM for 3 days with a medium change approximately every

24 hours. Vehicle control and appropriate positive controls were tested in parallel. Induction of CYP1A2, CYP2B6 and CYP3A4 was measured by mRNA expression assays selective for each CYP isoform by real time RT-PCR. Induction of in situ catalytic activity was determined for CYP1A2, CYP2B6, and CYP3A4 using specific probe substrates. Positive control inducers and solvent vehicle controls were included in the assays.

Results

Treatment with MYK-461 at concentrations of 0.05-15 µM resulted in no induction of CYP1A2 mRNA expression or activity but caused concentration-dependent induction of mRNA expression and enzyme activity of both CYP2B6 and CYP3A4 (Report #NC-19-0006), with maximal induction responses more than 2-fold and greater than 20% of positive control responses (Table 37). No significant morphology change was observed for all four donors at tested concentrations of MYK-461.

Table 37. Induction of drug metabolizing enzymes CYP isoforms by MYK-461

CYP enzyme		CYP1A2		CYP2B6		CYP3A4	
Hepatocyte Donor Lot	Endpoint	EC_{50} (µM)	E_{max}	EC_{50} (µM)	E_{max}[a]	EC_{50} (µM)	E_{max}
(b) (4)	mRNA	No induction observed		5.0 (0.26)	8.6	2.6 (0.38)	6.7[a]
	Activity			4.4 (0.35)	7.8	0.87 (0.074)	4.1 (0.085)
	mRNA			5.4 (0.32)	5.7	2.1 (0.38)	5.1[a]
	Activity			3.2 (0.31)	3.6	NA	NA
	mRNA			NA	NA	NA	NA
	Activity			NA	NA	1.4 (0.16)	9.6 (0.38)
	mRNA			5.0 (0.63)	6.0	1.8 (0.32)	16 (1.1)
	Activity			3.6 (0.40)	4.6	1.8 (0.63)	5.9 (0.72)

[a] SE not available due to E_{max} locked at observed maximal fold.
EC_{50} = Concentration at 50% of maximal induction effect
E_{max} = Maximal fold induction
Data are the mean (standard error) from 3 incubation wells.
Note: The positive control failed to induce CYP3A4 activity based on assay acceptance criteria for hepatocyte lot 348B; therefore, lot (b) (4) was employed as a replacement lot to assess activity.

A second study (NC-19-0012) assessed the potential of MYK-461 (0.015 to 20 µM) to induce CYP enzymes CYP3A4, CYP2C8, CYP2C9, and CYP2C19 in hepatocytes as measured by mRNA levels. In this study, MYK-461 at concentrations of 0.015 to 20 µM (4.1 to 5466 ng/mL) induced CYP2C8 mRNA expression in 2 out of 3 donors and induced CYP2C9 and CYP3A4 mRNA expression in all 3 donor lots. MYK-461 induction of CYP2C19 was evaluated in only 1 donor lot (Table 38). The study suggests that MYK-461 could have the potential to induce these enzymes in vivo. MYK-461 exhibited no cytotoxic effect and exhibited normal hepatic morphology up to 20 µM.

Table 38. Induction of drug metabolizing enzymes CYP isoforms by MYK-461

CYP Enzyme	EC_{50} (µM)	E_{max}	Comments
CYP2C8	2.88	3.25-fold	Induction observed in 2 out of 3 donors
CYP2C9	4.47	2.14-fold	Induction observed in all 3 donors
CYP2C19	3.55	2.25-fold	Induction assessed in only 1 donor
CYP3A4	3.94	7.70-fold	Induction observed in all 3 donors

EC50 = Concentration at 50% of maximal induction effect
Emax = Maximal fold induction

4.4. Excretion

4.4.1 Pharmacokinetics, distribution, metabolism, and excretion of [14C]MYK461 following oral or intravenous administration to male rats

This non-GLP study (#NC-19-0007, dated November 19, 2014) was conducted at (b) (4) The objective of the study was to evaluate the excretion of radioactive MYK-461 following a single oral or intravenous administration in rats. A similar study (Report #NC-19-0026) was conducted at a later date at (b) (4). The results of this study are generally consistent with the reviewed study.

Methods

The rate and extent of elimination of radioactivity were investigated in male intact SD rat following a single oral administration of 1 mg [14C]MYK-461/kg. Urine, feces, expired air, and cage washes were collected at intervals up to 120 hr post dose in separate glass containers. At the end of the study, animals were euthanized with an overdose of isoflurane anesthesia and the residual carcass from each animal was weighed and retained for radioanalysis. Following a single intravenous administration of 1 mg [14C]MYK-461/kg, the rate and extent of elimination of radioactivity were determined in bile duct-cannulated (BDC) male SD rat. Urine, feces, bile, and cage washes were collected at intervals up to 96 hr post dose in separate glass containers. Animals were euthanized with an overdose of isoflurane anesthesia and the residual carcass from each animal was weighed and retained for determination of radioactivity.

Results

Following a single oral dose of [14C]MYK-461, the mean total recovery of radioactivity was 96%. Radioactivity was eliminated rapidly, with approximately 77% of the administered radioactive dose recovered by 48 hr post-dose. The predominant route of elimination was through feces, which accounted for approximately 58% of the administered radioactivity. Urinary excretion accounted for approximately 23% of the administered dose. At 120 hr post-dose, approximately 7.6% of the administered dose was recovered in the residual carcasses (Table 39, Fig. 41).

Table 39. Recoveries of orally-administered [^{14C}]MYK-461

Oral (Group 1, Male Sprague Dawley Rat, 1 mg/kg)					
	% Mean Total Recovery	% Dose in Feces	% Dose in Urine	% Dose in Expired Air	% Dose in Residual Carcass
Bile duct-intact	95.8 ± 0.570	58.0 ± 1.25	22.6± 2.95	1.74 ± 0.390	7.63 ± 1.68

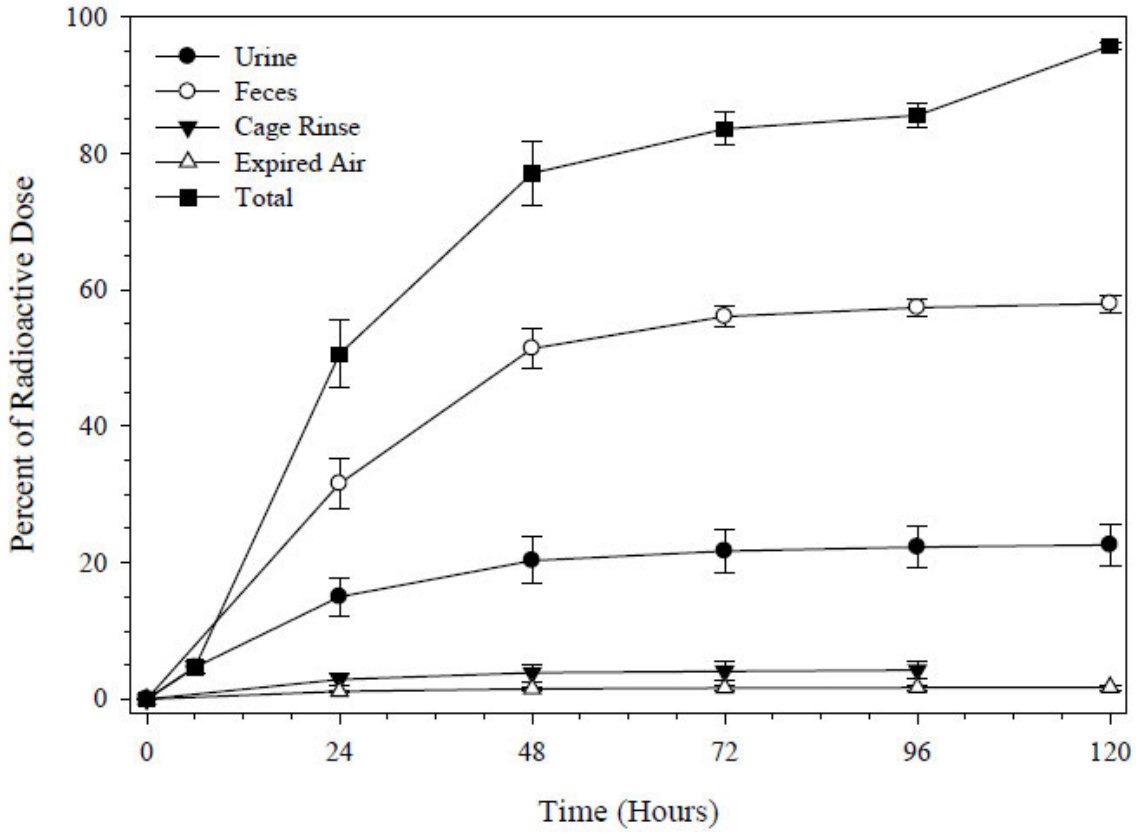


Figure 41. Mean cumulative percent of radioactive dose in urine, feces, cage rinse, and expired air at specified intervals after a single oral administration of [14C]MYK-461 to male Sprague Dawley rat. Note: Total includes urine, feces, cage rinse, cage wash, cage wipe, bile, bile cannula, jacket rinse, and carcass.

Following a single intravenous dose of [^{14}C]MYK-461 to bile duct-cannulated male rat, radioactivity was eliminated rapidly, with approximately 70% of the administered radioactive dose recovered by 48 h post-dose. Excretion in bile accounted for approximately 41% of the administered radioactivity and bile was the predominant route of elimination. Urinary and fecal excretion accounted for approximately 31% and 8% of the administered dose, respectively (Table 40, Fig. 42). At 96 h post-dose, approximately 7.9% of the administered dose was recovered in the residual carcasses. The mean total recovery of radioactivity was 94.0 ± 0.327%.

Table 40. Recoveries of intravenously administered [14C]MYK-461

Intravenous (Group 3, Male Sprague Dawley Rat, 1 mg/kg)					
	% Mean Total Recovery	% Dose in Feces	% Dose in Urine	% Dose in Bile	% Dose in Other[a]
Bile duct-cannulated	94.0 ± 0.327	7.59 ± 1.82	31.0 ± 14.0	41.1 ± 16.4	6.30

[a] Cage rinse, cage wash, cage wipe, residual carcass, bile cannula, and jacket rinse

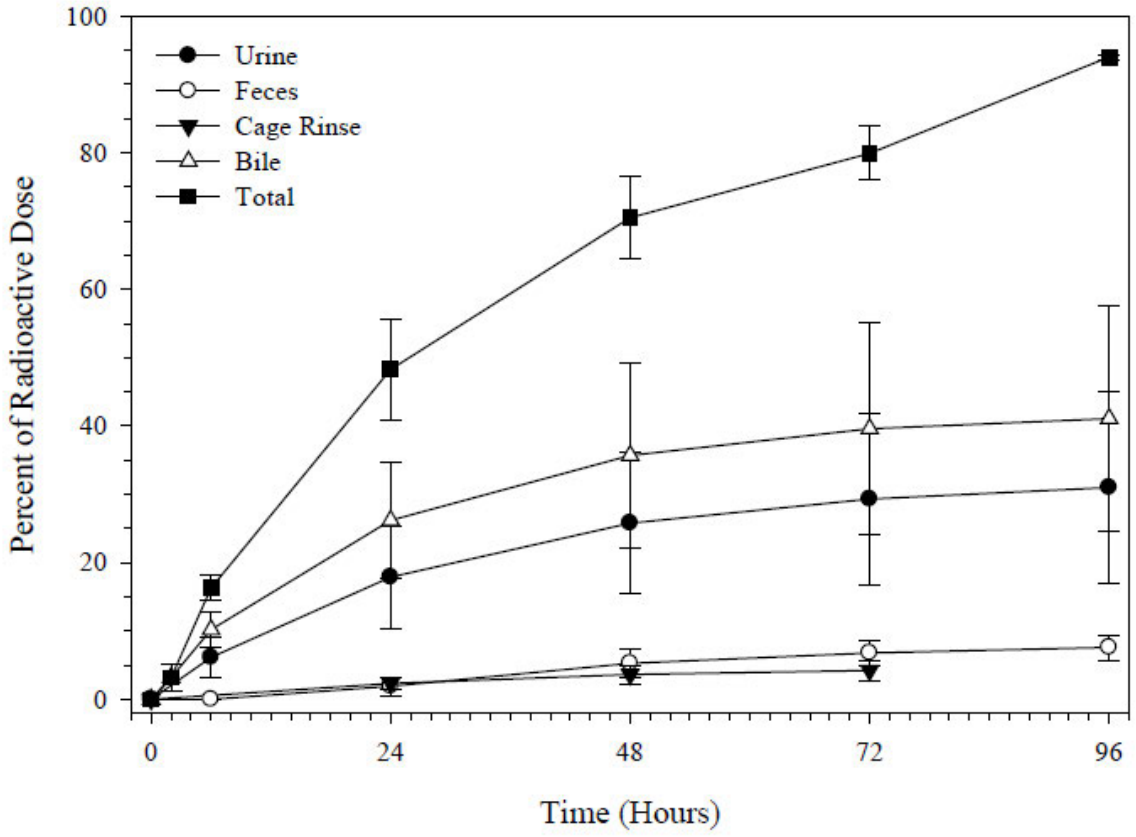


Figure 42. Mean cumulative percent of radioactive dose in urine, feces, cage rinse, and bile at specified intervals after a single intravenous administration of [14C]MYK-461 to male bile duct-cannulated Sprague Dawley rat.

6 General Toxicology

6.1 Single-Dose Toxicity

5.1.1 Exploratory tolerability of MYK-461 following a series of single oral gavage doses in dogs

This non-GLP exploratory study ((b) (4) 3-1168) was conducted by (b) (4) during October/November 2013. The objective of the study was to determine the tolerability of MYK-461 when administered orally as a series of single, escalating doses to male and female Beagle dogs.

Methods

Beagle dogs (n=4/sex) were randomized to receive one of the 4 escalating single oral doses (1 dog/sex/dose) of MYK-461 (0, 1.5, 4.5, 7.0 or 30 mg/kg) (Table 41). A minimum of 8 days was allowed between escalations. Dose formulations were prepared in 0.5% methylcellulose in reverse osmosis water and administered orally via gavage at a constant dose volume of 10 ml/kg.

Table 41. An overview of experimental design

Phase	MYK-461 Dose (mg/kg)	Dose Formulation Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals	
				Males	Females
1	1.5	0.15	10	1	1
2	4.5	0.45		1	1
3	7.0	0.7		1	1
4	30	3.0		1	1

Following dosing, animals were observed one hour post-dose and then daily for seven days. Body weights were recorded immediately prior to dosing and then for seven days following dose administration. At the end of the study, males receiving the first two low doses were returned to the testing facility colony. Females at all dose levels and males receiving the top 2 doses were euthanized on day 8. Plasma and muscle (flexor digitorum longus, extensor digitorum longus, left ventricle) samples were collected from these animals for determination of MYK-461 concentration.

Results

Acute oral administration of 1.5, 4.5 and 7 mg MYK-461/kg was well-tolerated by male and female dogs based on the absence of adverse clinical signs and the lack of definitive test substance-related effects on body weight. However, acute oral administration of 30 mg MYK-461/kg was not tolerated. Both male and female dogs were euthanized in moribund condition at approximately 1 hour post dose following clinical signs of extreme hypoactivity, unproductive vomiting (female only), and decreased capillary refill time (male only).

No dose-dependent increase in plasma MYK-461 concentrations (determined on day 8) for females receiving 1.5 mg/kg to 7.0 mg/kg was noted. On the other hand, a dose-dependent increase in tissue (both skeletal muscle and cardiac muscle) concentrations of test substance was noted over the dose range of 1.5 mg/kg to 7.0 mg/kg (Table 42).

Table 42. Plasma and tissue MYK-461 concentrations

Animal No.	Dose (mg/kg)	Admin. Date	Hours Post Dose	Mavacamten Concentration (ng/mL(g))			
				Soleus muscle	EDL	Left ventricle	Plasma
1F001	1.5	10/16/2013	>168 hr[a]	306	378	450	35.8
2F001	4.5	10/24/2013	>168 hr[a]	1080	859	992	56.1
3F001	7.0	11/4/ 2013	>168 hr[a]	1100	963	1260	53.7
3M001	7.0	11/4/ 2013	>168hr[a]	2710	1850	1850	88.9
4F001	30	11/18/2013	1.4 hr	5750	5950	19400	1780
4M001	30	11/18/2013	1.2 hr	3170	4140	12600	NA[b]

EDL= extensor digitorum longus muscle

[a] an exact time of sample collection was not recorded. Samples were collected on Day 8, following Day 1 dose administration.

[b] NA=Not applicable; plasma sample was not collected from animal 4M001.

6.2 Repeat-Dose Toxicity

6.2.1 One-Week exploratory oral toxicity study in wild-type RasH2 mice

A non-GLP study (#8367438, Sponsor reference #NC-17-0007.00 ((b) (4) DDO1383)) was conducted at (b) (4) between June 19, and July 13, 2017.

Male and female mice (CByB6F1-Tg[HRAS]2Jic (wild type)) were dosed MYK-461 (suspension in 0.5% methylcellulose in water) orally by gavage, once daily for 7 days. Dose formulation analysis confirmed dose levels at 5, 6.1 or 7 mg/kg/day instead of nominal concentrations of 6, 7 or 8 mg/kg/day, respectively (Table 43).

Table 43. Study design and dose levels

Group	Subgroup	No. of Animals Male	No. of Animals Female	Dose Level[a] (mg/kg/day)	Dose Concentration[b] (mg/mL)
1 (Control)	1 (Toxicity)	5	5	0	0
	2 (Toxicokinetic)	12	12		
2 (Mid)	1 (Toxicity)	5	5	7 (6.1)	0.7 (0.758)
	2 (Toxicokinetic)	30	30		
3 (High)	1 (Toxicity)	5	5	8 (7)	0.8 (0.7)
	2 (Toxicokinetic)	30	30		
4 (Low)	1 (Toxicity)	5	5	6 (5)	0.6 (0.5)
	2 (Toxicokinetic)	30	30		

a Nominal dose levels are listed first with actual dose levels, based on concentration and dose volume, listed in parentheses.

b Nominal dose concentrations are listed first with actual concentrations, based on dose analysis results, (listed in parentheses).

Results

MYK-461-related deaths occurred in animals receiving 7 or 8 mg/kg/day (actual concentration 6.1 or 7 mg/kg/day, respectively). A total of twelve animals receiving 8 mg/kg/day (7 mg/kg/day) were euthanized or were found dead between days 2 and 7. Death or moribund condition of these animals was attributed to inflammation and/or thrombosis in the left atrium of the heart. Ante-mortem clinical signs noted in one or more animals included hypoactivity, ataxia, hunched posture, thin, squinted eyes, irregular respiration, piloerection, sternal recumbency, and cold to the touch. A male receiving 7 mg/kg/day (6.1 mg/kg/day) was euthanized in a moribund condition on day 4. Prior to euthanasia, clinical signs consisting of hypoactivity, hunched posture, and piloerection were noted. All other animals survived to the terminal euthanasia. The high dose was associated with lower body weights and body weight loss relative to controls.

Test substance-related moderate increases in BUN relative to control were noted in 2 euthanized animals receiving 8 (7) mg/kg/day and in surviving animals administered ≥7 (6.1 actual) mg/kg/day (on day 8). High dose group animals showed higher ALT, AST and GDH than was control suggesting liver and, possibly muscle injury.
Test substance-related microscopic findings were noted in the heart, lung, spleen, and liver in early decedents and in a few animals survived to terminal euthanasia. Increased heart weight parameters in animals administered ≥6 (≥5 mg/kg/day actual) were correlated with multiple microscopic findings in the left atrium that consisted of minimal to mild myocardial degeneration/necrosis, mixed cell inflammation, thrombus, hemorrhage, and/or endocardial hyperplasia. In the lung, dose-related minimal to moderate vessel inflammation occurred in males administered ≥6 (≥5 actual) mg/kg/day and females administered 8 (7 actual) mg/kg/day. This finding correlated with increased lung weight parameters and was characterized by intramural and perivascular infiltrates of mononuclear cells predominantly affecting muscular arteries and arterioles. Minimally to moderately, dose-related increased extramedullary hematopoiesis in the spleen was noted in males receiving ≥7 (≥6.1 actual) mg/kg/day and females receiving ≥6 (≥5 mg/kg/day actual) and correlated with increased spleen weight parameters. In the liver, minimal or moderate degeneration/necrosis of hepatocytes, moderate coagulative necrosis, mild or moderate thrombosis in sinusoids, and/or mild or moderate vacuolation of centrilobular hepatocytes were observed in high dose animals. These findings correlated with decreased liver/gallbladder weight parameters. Low thymus and kidney weights with no histopathologic correlates occurred in high dose males. A NOAEL was not found in this study.

Plasma concentrations of test substance reached maximum between 0.5 and 1 hr post dose on days 1 and 7 in all treated groups. Exposures to MYK-461 increased with increasing dose. Accumulation of test substance was noted with repeated dosing at all dose levels. No sex-dependent differences were noted (Table 44).

Table 44. Summary of toxicokinetics after single and 7 doses of MYK-461 administration in mice

	Dose	C_{max} (ng/mL)		t_{max} (h)		AUC_{0-t} (ng·h/mL)		AUC_{0-24} (ng·h/mL)	
Sex	(mg/kg/day)	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Male	7.0 (6.1) *	5720	7440	0.5	0.5	66500	96300	66500	96300
	8.0 (7.0) *	6060	8880	0.5	1.0	75600	138000	75600	138000
	6.0 (5.0) *	3360	5850	0.5	0.5	40500	73200	40500	73200
Female	7.0 (6.1) *	5280	7500	1.0	0.5	61000	83500	61000	83500
	8.0 (7.0) *	6060	8100	0.5	1.0	82500	107000	82500	107000
	6.0 (5.0) *	4040	5160	1.0	0.5	48100	68500	48100	68500

Values were rounded to 3 significant figures (1 decimal place for t_{max})
* = Nominal doses are listed first, with actual doses listed in parenthesis.

6.2.2 One-month oral range-finding toxicity study in wild-type RasH2 mice

Conducting laboratory and location: (b) (4)

Sponsor Study number: NC-17-0013.00, DDC0139
Testing Facility Study Number: 8368215
Date of study initiation: July 27, 2017
Date of last necropsy: September 28, 2017
Drug, Lot number: MYK-461, 150030, 99.98% purity
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

Following dosing on day 1 at 6 mg MYK-461/kg, 3 toxicity males and 3 TK males were found dead or euthanized in a moribund condition. The cause of death of 2 males were related to microscopic findings of kidney tubular degeneration/necrosis. The cause of death of other animals was undetermined but was likely test substance related. Additionally, another TK male that received a single dose of 6 mg/kg/day and given 8 dosing holidays was found dead on day 8. These animals showed poor clinical condition including hypoactivity. Thus, the dose was lowered to 5 mg/kg/day from day 3 onward. In animals survived until scheduled necropsy, decrease ($P < 0.05$) in body weight gain was noted at all doses for males for days 1 through 28. Absolute and relative heart weights were dose-dependently increased ($P < 0.05$) at ≥4 mg/kg/day. The NOAEL was 2 mg/kg/day for female mice and there was no NOAEL for male mice.

Purpose

The primary objective of the study was to evaluate the toxicity, toxicokinetic profile and no observed adverse effect level of MYK-461 when administered orally to mice, once daily, for 28 days.

Methods

Formulation

Test substance was suspended in 0.5% methylcellulose in reverse osmosis water. Drug formulations were prepared once and stored in refrigerator before use (stable for at least 56 days). Each of the prepared dosing formulations were evaluated for concentration on days 1, 3 and 29 of the dosing phase.

Animals

Species/Strain: RasH2 (001178-W [wild type], CByB6F1-Tg[HRAS]2Jic) mice from (b) (4)

#/Sex/Group: 12; and 30 (12 for the control) for the toxicokinetics study (Table 45)

Age: 11-12 weeks old at start of dosing

Weight: Males: 25.1 to 38.4 gm; Females: 18.6 to 27 gm at the start of dosing

Husbandry: Animals were housed in groups of 3 per cage. Food and water were available *ad libitum* throughout the study period.

Dosing

Doses: Animals were randomly assigned to four dose groups. Both main study and TK groups received test substance at doses of 2, 4 or 6 mg/kg/day (Table 45) for a targeted duration of at least 28 days. As a result of mortality and adverse effects observed on day 1 at 6 mg/kg/day, dosing was suspended on day 2 and resumed on day 3 at 5 mg/kg/day (see footnote to Table 45). Dose levels were based on the results of a previous 1-week oral toxicity study (see section 6.2.1) in mice of the same strain in which test substance was administered at nominal doses of 6, 7 or 8 mg/kg/day. Treatment at 7 or 8 mg/kg/day (actual dose concentration of 6.1 or 7 mg/kg/day, respectively) resulted in hypoactivity and moribundity/mortality of animals in both groups. Test substance-related microscopic findings were noted in the heart, lung, spleen, and liver in animals administered ≥6 mg/kg/day (≥5 mg/kg/day actual). A NOAEL was not identified in this study.

Table 45. Study design and dose levels as of days 3 to 28

Group[a]	Subgroup	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration[b] (mg/mL)
		Male	Female		
1 (Control)	1 (Toxicity)	12	12	0	0
	2 (Toxicokinetic)	12	12		
2 (Low)	1 (Toxicity)	12	12	2	0.2
	2 (Toxicokinetic)	30	30		
3 (Mid)	1 (Toxicity)	12	12	4	0.4
	2 (Toxicokinetic)	30	30		
4 (High)	1 (Toxicity)	12	12	5	0.5
	2 (Toxicokinetic)[c]	29[d]	29[d]		

As a result of toxicity observed on Day 1 at 6 mg/kg/day, Group 4 had a dosing holiday on Day 2. Dosing resumed on Day 3 at a lower dose level, 5 mg/kg/day.

a Group 1 was administered vehicle control article only.

b Dose concentrations were based on test article as supplied. No correction factor was used.

c The 4th and 5th three toxicokinetic animals/sex in Group 4, Subgroup 2 had an 8-day washout period after Day 1 and were dosed again on Day 9 of the dosing phase.

d The total number of animals assigned to Group 4, Subgroup 2, was 38. However, the number reflected in this table was 29, since that was the number of animals assigned to Group 4, subgroup 2 as of Day 3 of the dosing phase.

Observations and Measurements

Clinical Signs, Body Weights, Food Consumption, and clinical pathology.

Terminal procedures: Necropsy, organ weights, gross pathology (macroscopic examination of abnormalities and collection of tissues from an extensive list on all

main study group animals and early decedents). Microscopic examination was performed on all tissues collected from all main study animals in the control and the high dose group animals and on all toxicity animals that died or were euthanized at an unscheduled interval.

Results

Analysis of Formulations: The achieved concentrations of MYK-461 for all doses were in the range from 88.7 to 104.4% of nominal.

Mortality: Following dosing on day 1, 3 toxicity males (#M0306, M0307, and M0308) and three TK males (#M0319, M0336, and M0341) receiving 6 mg/kg/day were found dead or euthanized in a moribund condition. Prior to death or euthanasia, clinical signs consisting of sternal recumbency, hypoactivity, hunched posture, piloerection, low carriage, pale body, labored respiration, opaque and/or squinting eyes, and cold to touch were noted. Clinical pathology changes in blood samples collected from #M0306 included mildly increased urea nitrogen, creatinine, and phosphorus concentrations, supportive of renal azotemia, which correlated with kidney tubular degeneration/necrosis, and mildly to moderately increased AST, ALT, and AP activities, which were suggestive of hepatobiliary alteration but lacked microscopic correlates. The cause of death of two males (#M0306, M0307) was related to the microscopic finding of kidney tubular degeneration/necrosis. The cause of death of remaining animals was undetermined but was MYK-461-related. Additionally, a TK male (#M0332) that received 6 mg/kg/day on day 1 and scheduled for 5 mg/kg/day dosage beginning on day 9 was found dead on day 8. It was hypoactive with low carriage and lost 1 gm body weight for the past 1 week. The cause of death was not ascertained. As a result of toxicity observed on day 1 at 6 mg/kg/day, dosing was suspended on day 2 for this group and resumed on day 3 at 5 mg/kg/day.

Clinical Signs: As described above, clinical signs were noted on day 1 in the high dose group early decedent males. No test substance-related clinical signs were noted in surviving animals after reducing the top dose to 5 mg/kg/day from day 3.

Body Weights: A statistically significantly decrease in body weight gain relative to control was noted at all doses for males for days 1 through 28. The decreased body weight gain was the result of weight loss that occurred during weeks 1 and 2 ($P < 0.05$) and was generally correlated with low food consumption. The mean body weights in weeks 3 and 4 were not statistically significantly different from control (Table 46).

Food Consumption: Food consumption was low during days 1 to 8 for males receiving 4 or 6/5 mg/kg/day. This correlated with the body weight loss that occurred during the first 2 weeks.

Table 46. Group mean body weight change in mice dosed with MYK-461

Test Article	(dosage)	1	2	3	4
MYK-461	mg/kg/day	0	2	4	6/5

Data Presented in "g" Interval X through X DSNG

Group/ Subgroup/ Sex	Phase / Day	1 - 8	8 - 15	15 - 22	22 - 28	1 - 28
1/1/M	Mean	0.4	0.3	0.9	0.7	2.2
	SD	0.38	0.64	0.51	0.65	1.18
	N	12	12	12	12	12
	P(overall)	<0.0001	0.0013	0.1104	0.9399	<0.0001
2/1/M	Mean	-1.3*	0.4	0.7	0.8	0.6*
	SD	1.26	0.32	0.37	0.68	1.16
	N	12	12	12	12	12
	P(v1)	<0.0001	0.8868	-	-	0.0008
3/1/M	Mean	-0.4*	-0.2*	0.8	0.7	0.9*
	SD	0.52	0.38	0.35	0.44	0.92
	N	12	12	12	12	12
	P(v1)	0.0054	0.0294	-	-	0.0063
4/1/M	Mean	-0.7*	-0.3*	0.4	0.6	0.0*
	SD	0.45	0.44	0.44	0.44	0.68
	N	9	9	9	9	9
	P(v1)	0.0003	0.0195	-	-	<0.0001
	Statistics	AT	AT	A	A	A

* P<=0.05
A = ANOVA and Dunnett's
T = Rank-transformed data

Clinical Pathology: No test substance-related effects were noted in scheduled euthanized animals. Blood samples collected from an unscheduled euthanized high dose male (M0306) showed increased concentrations of BUN, creatinine, phosphorus, AST, ALT, and AP. Blood samples taken from both moribund males (#M0306, M0308) showed lower total protein, albumin, and calcium concentrations and mildly lower albumin: globulin ratio relative to control animals.
Organ Weights: At terminal euthanasia, increases in mean absolute and relative heart weights were noted for animals receiving ≥4 mg/kg/day relative to control values with no macroscopic or microscopic correlates. Absolute and relative heart weights were dose-dependently increased reaching statistical significance at ≥4 mg/kg/day (Table 47). The increase in heart weight was attributed to myocardial hypertrophy, a compensatory response to negative inotropy.
Gross Pathology: No macroscopic findings were noted.
Histopathology: No test substance-related microscopic observations were noted at the terminal euthanasia. Two early decedents showed marked kidney tubular degeneration/necrosis, which correlated with changes in the clinical pathology results. According to the sponsor, the NOAEL was 4 and 5 mg/kg/day for male and female mice, respectively. The reviewing pharmacologist disagrees with this assessment. Based on decreased body weight gain (a 59% to 100% decrease relative to control group in male mice at ≥2 mg/kg/day) and increased heart

weight (>10% increase in absolute or relative heart weight in female mice at ≥4 mg/kg/day), the NOAEL was 2 mg/kg/day for female mice and there was no NOAEL for male mice.

Table 47. Group mean heart weights (absolute and relative to body and brain weights) in mice dosed with MYK-461

	MYK-461							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	2	4	5/6	0	2	4	5/6
Heart								
Absolute Weight (g)	0.1525	104	124*	133*	0.1254	106	112*	116*
Body Weight Ratio (%)	0.4672	107	129*	142*	0.5034	107	119*	119*
Brain Weight Ratio (%)	31.3493	106	127*	139*	25.4125	106	113*	117*

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for test article-treated groups are expressed as percentage control mean value.

Toxicokinetics: Following oral administration of MYK-461 at dose levels 2, 4 or 6 mg/kg/day, plasma concentrations were at maximum between 0.5 and 2 h post dosing. Systemic exposures (Cmax and AUC) increased more than the proportionate dose increment on all days of measurement. There was no sex-dependent difference in the systemic exposure. Limited accumulation was noted after repeated daily oral doses (Table 48).

Table 48. Summary of toxicokinetic parameters of MYK-461 in mice treated orally with MYK-461 for 28 days

Sex	Dose Level (mg/kg/day)	Day	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{0-24} (ng·h/mL)	Accumulation Ratio C_{max}	Accumulation Ratio AUC_{0-24}	Sex Ratio C_{max}	Sex Ratio AUC_{0-24}
Male	2	1	1350	1.0	24.0	17000	NA	NA	NA	NA
		28	2050	0.5	24.0	25900	1.52	1.53	NA	NA
	4	1	2840	0.5	24.0	34000	NA	NA	NA	NA
		28	4930	0.5	24.0	69600	1.74	2.05	NA	NA
	5	9	4710	1.0	24.0	55300	NA	NA	NA	NA
		28	6370	2.0	24.0	99800	1.35	1.81	NA	NA
Female	2	1	1540	1.0	24.0	19200	NA	NA	1.14	1.13
		28	1840	0.5	24.0	22600	1.20	1.17	0.899	0.872
	4	1	4000	0.5	24.0	40900	NA	NA	1.41	1.20
		28	4510	0.5	24.0	54100	1.13	1.32	0.915	0.778
	5	9	5700	0.5	24.0	58800	NA	NA	1.21	1.06
		28	5380	0.5	24.0	61900	0.944	1.05	0.845	0.620

NA - Not applicable
Values are rounded to 3 significant figures (1 decimal place for t_{max} and t_{last}).
AUC_{0-t} values are equivalent to AUC_{0-24} values and are therefore not shown.
Due to toxicity observed on Day 1, samples were not collected after 2 hours post dose following administration of 6 mg/kg/day. The non-sampled TK animals scheduled for sampling on Day 1, administered 6 mg/kg/day once, were not dosed from Day 2 to Day 8, and were dosed once at 5 mg/kg/day on Day 9, together with 8 additional TK animals/sex. The TK animals scheduled for sampling on Day 28, administered 6 mg/kg/day once on Day 1, were not dosed on Day 2, and resumed dosing at 5 mg/kg/day on Day 3.
The dosing duration was 26 days for the 5 mg/kg/day dose level.

6.2.3 6-Week oral toxicity study in rats followed by a 4-week recovery period

Conducting laboratory and location: (b) (4)

Sponsor Study number: NC-15-0008
Testing Facility Study Number: 14-2402
Date of study initiation: March 26, 2014
Date of last necropsy: June 19, 2014
Drug, Lot number: MYK-461, 400-13-01-53, 99.6% purity
GLP compliance: Yes
QA statement: Yes, signed

Key Study Findings

Daily oral administration of 3 mg MYK-461/kg resulted in moribundity/mortality of a male and a female rat on day 7/8. Additionally, a female and a male were found dead on days 24 and 34, respectively. These animals exhibited rapid/labored breathing, decreased activity, piloerection, pallor and thinness. The major factor contributing to death for all unscheduled decedents was cardiac toxicity resulting in heart failure. Microscopically, myocardial hypertrophy, myocardial degeneration and/or inflammation, endocardial degeneration/necrosis, atrial thrombosis, and/or ventricular dilatation were present in the hearts of both unscheduled and scheduled necropsies. Partial recovery was noted for heart findings. Additional microscopic findings included centrilobular necrosis, centrilobular congestion, and/or diffuse hepatocellular vacuolation in the liver, congestion, edema, and/or increased numbers of alveolar macrophages in the lungs; and pancreatic edema were noted in all unscheduled decedents and a few terminal sacrificed rats. The findings were absent in the recovery animals. Based on the histopathological findings, the NOAEL was 1 mg/kg/day.

Purpose

The primary objective of the study was to evaluate the toxicity, toxicokinetic profile and no observed adverse effect level of MYK-461 when administered orally to rats, once daily, for 6 weeks, and to estimate the potential for reversal of any toxic effects within a 4-week drug-free period.

Methods

Formulation

Test substance was dissolved in 0.5% methylcellulose in distilled water. Drug formulations were prepared weekly and stored at room temperature. The prepared dosing formulations were evaluated for homogeneity (week 1) and concentration on the day of dose preparations for weeks 1 and 6. Analyses of the formulations demonstrated that MYK-461 in 0.5% Methylcellulose in distilled water was stable when stored at ambient temperature for 10 days.

Animals

Species/Strain: Rat, Sprague-Dawley CD Rats from (b) (4)

#/Sex/Group: 15 for the control and high dose groups (5 in each group were designated for the recovery phase), 10 for mid and low dose groups; and 6 (3 for the control) for the toxicokinetics study (Table 49)

Age: 9 weeks old at start of dosing

Weight: Males: 283 to 328 gm; Females: 176 to 217 gm at the start of dosing

Husbandry: Animals were housed one in each cage. Food and water were available *ad libitum* throughout the study period except during the blood collection periods for clinical pathology and necropsy where food was withdrawn.

Dosing

Doses: Animals were randomly assigned to four dose groups. Both main study and TK groups received test substance at doses of 0.3, 1 or 3 mg/kg/day (Table 49) for 6 consecutive weeks. Dose levels were based on the results of a previous exploratory 14-day oral toxicity study (# (b) (4) 13-1045) in rats of the same strain in which test substance was administered at doses of 1, 3 or 10 mg/kg/day. Treatment at ≥ 3 mg/kg/day caused adverse cardiac and hepatic effects. Test substance related microscopic findings in the heart consisted of acute or subacute inflammation that was present primarily in subendothelial, perivascular, or epicardial tissue of the atrium with lesser involvement of the ventricle and atrioventricular dilation. Findings in the liver consisted of centrilobular hepatocellular necrosis, centrilobular hepatocellular vacuolation, and centrilobular congestion. The high dose was not tolerated, resulting in mortality/moribundity within the first week of the dosing period.

Table 49. Study design

Group	Treatment	Dose (mg/kg/day)[a]	Number of animals					
			Main study		Recovery phase		Satellite study[b]	
			Male	Female	Male	Female	Male	Female
1	Control	0	10	10	5	5	3	3
2	MYK-461	0.3	10	10	0	0	6	6
3	MYK-461	1	10	10	0	0	6	6
4	MYK-461	3	10	10	5	5	6	6

[a]Doses represent the active ingredient.
[b]Satellite animals used for toxicokinetic blood sampling only.

The last 5 surviving rats/sex in groups 1 and 4 of the main study were designated for the recovery phase during which the rats were not dosed.

Mode and Duration of Administration: Once daily orally by gavage for all groups for 6 weeks. Control animals received drug vehicle (5 ml/kg). Recovery group (1

and 4) animals were observed for an additional 4 weeks after administration of the last dose.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for mortality and clinical signs.

Body Weights: Recorded twice pretest, weekly during the drug phase and in the recovery phase and terminally (after fasting and just prior to necropsy) for all rats.

Food Consumption: Recorded pretest, and weekly during the drug treatment and in the recovery phase. For toxicokinetic animals, food consumption was measured during the pretest period only.

Ophthalmoscopy: Conducted on all animals pretest and in week 6 in main study and recovery phase animals.

Hematology[1] and Clinical Chemistry[2]: Blood samples were collected from vena cava from all main study animals (10/sex/group) at the end of treatment period and from recovery group animals (5/sex/group) at the end of the recovery phase. Animals were fasted overnight and anesthetized with isoflurane before blood sampling. Blood was not collected in pretest period.

Urinalysis[3]: Urine samples were collected overnight from main study fasted animals at the end of treatment period and from recovery group fasted animals at the end of the recovery period.

Gross Pathology: A complete necropsy including macroscopic examination of abnormalities and collection of tissues from an extensive list (Table 50) was done on all main study and recovery group animals fasted overnight at study termination.

Organs Weighed: The terminal body weights and weights of the organ collected (adrenal, brain, heart, prostate, seminal vesicles, epididymides, kidneys, liver, pituitary, ovaries, uterus, spleen, testes, thymus and thyroid) were recorded for all surviving main study and recovery group animals at study termination at the scheduled necropsy.

Histopathology: Microscopic examination was performed on all tissues listed in Table 50 from all main study animals in the control and the high dose groups sacrificed at the end of treatment and recovery periods. Additionally, all gross lesions and target organs (heart, liver and lung) were examined for all dose group main study animals sacrificed at termination.

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[1] erythrocytes, hematocrit, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, red cell distribution width, reticulocytes, thrombocytes, white blood cell, white blood cell differential, activated partial thromboplastin time, prothrombin time

[2] ALT, AST, AP, Sorbitol dehydrogenase, total bilirubin, total protein, albumin, glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, cholesterol

[3] pH, specific gravity, volume, bilirubin, occult blood, glucose, ketone, protein, sediment microscopy

Table 50. Tissues/organs sampled for histopathological examination

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY (Groups 1 and 4)
adrenal glands	X	X	X
animal identity		X	
aorta (thoracic)		X	X
bone marrow smear (femur)		X	
bone (sternum, femur with joint)		X	X
bone marrow (sternum, femur)		X	X[a]
brain (medulla, pons, cerebrum and cerebellum)	X	X	X
diaphragm		X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
Harderian gland		X	X
heart	X	X	X[f]
kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X[b]
liver	X	X	X[f]
lungs (with mainstem bronchi)		X	X[f]
lymph nodes (mesenteric, axillary)		X	X
mammary gland (inguinal)		X	X[c]
nerve (sciatic)		X	X
optic nerve		X	
ovaries	X	X	X
pancreas		X	X
pituitary gland	X[d]	X	X
prostate gland	X[e]	X	X
salivary glands (submandibular)		X	X
seminal vesicles	X[e]	X	X
skeletal muscle (*rectus femoris, quadriceps femoris, longissimus dorsi-posture*)		X	X
skin (dorsal – base of tail)		X	X
small intestine (duodenum, ileum, jejunum, Peyer's patches/GALT)		X	X[c]
spinal cord (cervical, thoracic, lumbar)		X	X

spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X[d]	X	X
tongue		X	X
trachea		X	X
urinary bladder		X	X
uterus (body/horns) with cervix	X	X	X
vagina		X	X
Zymbal's gland		X	
tissues with macroscopic findings including tissue masses		X	X[f]

a: Qualitative examination (no differential count).
b: Cecum and colon only; rectum was not examined.
c: Mammary gland for males and GALT were evaluated only if present in routine sections.
d: Weighed post-fixation.
e: Prostate and seminal vesicles were weighed together.
f: Tissues with macroscopic findings and target organs were also examined for animals in Groups 2 and 3

Toxicokinetics: Blood samples for test substance determination were collected prior to dosing (week 6 only), and 0.5, 1, 2, 4, 8 and 24 hr (prior to 2nd day dosing) after dosing on study days 1 and 41 (week 6) from the jugular vein from all non-fasted, unanesthetized TK animals (3 rats/sex/time point). All TK animals were euthanized, and the carcasses were discarded after blood collection. Blood samples from control animals were collected at one time point (1 hr post dose) only.

Results

Analysis of Formulations: The achieved concentrations of MYK-461 for all doses, prepared in weeks 1 and 6 were within target and ranged from 92.6% to 101.7% of nominal. The preparations produced homogeneous mixtures.

Mortality: Two males and two females receiving 3 mg/kg/day were euthanized or found dead (Table 51).

Table 51. Mortality

Dose (3 mg/kg/day)	Group	Animal # and sex	Mortality details
3	Main study	4055, Male	Found dead on day 7. Rapid breathing noted on the day 6. Histopathologically, multifocal or diffuse acute to subacute myocardial inflammation of the atria was noted. Liver exhibited moderate to marked centrilobular congestion, necrosis, diffuse hepatocellular vacuolation and Kupffer-cell hyperplasia.
3	Toxicokinetics	4588, Female	Euthanized after dosing on day 8 for welfare reasons. Exhibited decreased activity, rapid breathing, piloerection, skin pallor and thinness.
3	Recovery	4584, Female	Found dead on day 24. No clinical signs observed on the day preceding its demise. Histopathologically, multifocal or diffuse acute to subacute myocardial inflammation of the atria was noted.
3	Main study	4059, Male	Found dead on day 34. Labored breathing and piloerection were observed before this animal met its demise. Microscopically, bilateral atrial thrombosis and ventricular dilatation was present. Liver exhibited moderate to marked centrilobular congestion, necrosis, diffuse hepatocellular vacuolation and Kupffer-cell hyperplasia.

Clinical Signs: Animals euthanized prematurely or found dead exhibited rapid/labored breathing, piloerection, decreased activity, skin pallor and/or thinness. No test substance-related clinical signs were noted in animals that survived until their scheduled necropsy.
Body Weights: No effect on body weights.
Food Consumption: No test substance-related effects on food consumption.
Ophthalmoscopy: No significant changes
Hematology: No test substance-related effects.
Clinical Chemistry: A female (#4574) receiving 3 mg/kg/day was the lone animal that exhibited marked increases in AST, ALT, BUN, creatinine and phosphorus, and decrease in total proteins. These findings correlated with microscopic observation of myocardial degeneration and hypertrophy and hepatocellular centrilobular necrosis and diffuse vacuolation.
Urine analysis: No effect.
Gross Pathology: As noted above, marked enlargement of the heart was noted in a high dose female (#4574). Abnormal contents (thin red or yellow fluid) in the thoracic cavity in two unscheduled males and one unscheduled female and pancreatic edema in one unscheduled male. The edematous pancreas correlated

with pancreatic edema microscopically. No macroscopic findings were noted at the end of the 4-week recovery period.

Organ Weights: Treatment-related statistically significant increase in heart weight (absolute and relative to body and brain weights) relative to control was noted in high dose females with histologic correlates. Additionally, a non-dose-dependent increase in thyroid/parathyroid weights (absolute and relative to body and brain weights) relative to control was noted in males and females given ≥1 mg/kg/day with no histologic correlates (Table 52). The sponsor considers this change was not an adverse effect. A partial recovery with an increased heart weight was noted for high dose females (Table 53).

Table 52. Group mean organ weights (% difference relative to controls) in rats dosed with MYK-461 for 6 weeks

Group/sex Dose (mg/kg/day)	2M 0.3	3M 1	4M 3	2F 0.3	3F 1	4F 3
Heart						
Absolute weight (%)	-	-	-	-	-	52[a]
vs. body weight (%)	-	-	-	-	-	57[a]
vs. brain weight (%)	-	-	-	-	-	57[a]
Thyroids/Parathyroid Glands						
Absolute weight (%)	-	28[a]	21[a]	-	20[a]	20[a]
vs. body weight (%)	-	22[a]	20[a]	-	23[a]	25[a]
vs. brain weight (%)	-	29[a]	23[a]	-	24[a]	25[a]

[a]Statistically significant difference between mean values for test article-treated and control groups.

- = not test article-related.

Table 53. Group mean organ weights (% difference relative to controls) in rats dosed with MYK-461 for 6 weeks and following 4-week recovery phase

Group/sex Dose (mg/kg/day)	4M 3	4F 3
Heart		
Absolute weight (%)	-	17[a]
vs. body weight (%)	-	21[a]
vs. brain weight (%)	-	9[a]
Thyroids/Parathyroid Glands		
Absolute weight (%)	10	-
vs. body weight (%)	17	-
vs. brain weight (%)	9[a]	-

[a]Statistically significant difference between mean values for test article-treated and control groups.

- = not test article-related.

Histopathology: Test substance-related findings were noted in the heart, liver, lungs and pancreas of males and females at 3 mg/kg/day. In the heart, histopathologic changes were noted in 5 male and 4 female terminal sacrificed animals and in 2 males and a female sacrificed prematurely (Table 54). The most severely affected hearts exhibited sub-gross enlargement (myocardial hypertrophy), myocardial degeneration, cartilaginous/osseous metaplasia of the chorda tendineae, endocardial degeneration/inflammation, and valvular inflammatory-cell infiltrate/inflammation. In unscheduled decedents (male #4055; female #4584), multifocal or diffuse acute to subacute myocardial inflammation of the atria was noted in addition to those listed above for the scheduled sacrifice animals. Another unscheduled decedent male (#4059) showed bilateral atrial thrombosis and ventricular dilatation, which was not noted in scheduled sacrifice animals. Also, sub-gross enlargement (myocardial hypertrophy), characterized by an increase in thickness of the muscular portions of the heart, was present in the left ventricle and interventricular septum and in all heart segments (atria, ventricles, papillary muscles, and septa) in both scheduled and 2 unscheduled decedents (#4055 and #4584). Myocardial degeneration consisted of focal or multifocal loss of myofibers associated with a myxomatous interstitial appearance and infiltration of a few mononuclear-cell inflammatory cells. This was present primarily in the apex and left ventricle wall with lesser involvement of papillary muscles and atria. Inflammation of valves (including thickness of valve leaflets) predominantly left atrioventricular valve and to some extent right atrioventricular valve was noted in 2 males and 2 females of unscheduled sacrifice. Endocardial degeneration was primarily in the left atrium. At the recovery phase, minimal multifocal myocardial degeneration in the apical portion of the left ventricular wall and associated papillary muscle was noted in one male suggesting partially recovery of most heart findings.

In the liver, moderate to marked centrilobular congestion, necrosis, diffuse hepatocellular vacuolation and Kupffer-cell hyperplasia was noted in the 2 unscheduled decedent males, the unscheduled female (#4584) and a terminal sacrificed female. In the lungs, 2 terminal sacrifice females, 2 unscheduled males and an unscheduled sacrifice female (#4584) exhibited increased alveolar macrophages, perivascular edema and congestion. Additionally, pancreatic edema was noted in 2 unscheduled decedent males. According to the sponsor, liver, lungs, and pancreas findings are attributed to decreased cardiac function/output and/or heart failure and are not considered directly test article related. Based on the histopathological findings, the sponsor suggests a NOAEL of 1 mg/kg/day for both sexes.

Table 54. Test article-related microscopic findings in the heart in rats dosed with MYK-461 for 6 weeks

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg/kg/day)	0	0.3	1	3	0	0.3	1	3
Number of animals	10	10	10	10[a]	10	10	10	11[b]
Enlargement, Subgross (Hypertrophy, Myocardial)								
minimal	0	0	0	0	0	0	0	2
slight	0	0	0	2	0	0	0	0
moderate	0	0	0	0	0	0	0	1
marked	0	0	0	0	0	0	0	2
total	0	0	0	2	0	0	0	5
Degeneration, Myocardial								
minimal	0	0	0	2	0	0	0	2
slight	0	0	0	2	0	0	0	2
total	0	0	0	4	0	0	0	4
Cartilaginous/Osseous Metaplasia, Chorda Tendineae								
slight	0	0	0	1	0	0	0	2
total	0	0	0	1	0	0	0	2
Degeneration/Inflammation, Endocardial								
slight	0	0	0	1	0	0	0	3
moderate	0	0	0	1	0	0	0	0
total	0	0	0	2	0	0	0	3
Inflammatory Cell Infiltrate/Inflammation, Valvular								
minimal	0	0	0	1	0	0	0	2
slight	0	0	0	1	0	0	0	0
total	0	0	0	2	0	0	0	2
Inflammation, Myocardial								
minimal	0	0	0	1	0	0	0	0
slight	0	0	0	0	0	0	0	1
total	0	0	0	1	0	0	0	1
Thrombus, Atrial								
marked	0	0	0	1	0	0	0	0
total	0	0	0	1	0	0	0	0
Dilatation								
moderate	0	0	0	1	0	0	0	0
total	0	0	0	1	0	0	0	0
Proliferation, Endocardial								
slight	0	0	0	0	0	0	0	1
total	0	0	0	0	0	0	0	1
Proliferation, Epicardial								
slight	0	0	0	0	0	0	0	1
total	0	0	0	0	0	0	0	1
Number of tissues examined	10	10	10	10	10	10	10	11

[a] Includes 2 unscheduled decedents

[b] Includes 1 unscheduled decedent (originally assigned to recovery sacrifice)

Toxicokinetics: Systemic exposures increased approximately proportionately to the increase in dose on day 1 and day 41 in both sexes. Although there was no sex-dependent difference, the systemic exposure of female rats to MYK-460 (and not MYK-461) was generally higher than that of males. Accumulation was noted after repeated daily oral doses. Based on AUC values, the accumulation ratio was in the range of 1.7 to 2.8, and it tended to be higher in males than in females (Table 55). There was no evidence of meaningful interconversion of MYK-461 to its enantiomer, MYK-460, after either single dose or repeat-dose administration.

Table 55. Summary of MYK-461 exposure data in a 6-week oral study in rats

Dose (mg/kg/day)	C_{max} (ng/mL)				AUC_{0-24} (ng·h/mL)			
	Day 1		Week 6		Day 1		Week 6	
	M	F	M	F	M	F	M	F
MYK-461								
0.3	28.0	33.9	60.5	62.3	442	553	1030	1000
1	108	124	307	283	1780	2270	4940	4810
3	323	475	726	919	5790	8740	12400	15200
MYK-460								
0.3	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
1	0.385	0.543	0.588	0.888	0.578	2.07	2.71	9.35
3	1.15	1.58	1.29	3.20	13.2	22.6	18.6	32.6

M = male(s); F = female(s); BLQ = below the assay limit of quantitation (0.250 ng/mL)

6.2.4 Three-month oral toxicity study in rats followed by a 4-week recovery period

Conducting laboratory and location: [redacted] (b) (4)

Sponsor Study number: NC-19-0050
Testing Facility Study Number: TXC1549
Date of study initiation: April 21, 2015
Date of last necropsy: August 18, 2015
Drug, Lot number: MYK-461, 15B0013 ([redacted] (b) (4) lot 150030), 99.87% purity
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

Daily oral administration of 2 mg MYK-461/kg resulted in moribundity/mortality of a male and 3 female rats on study day 10. These animals exhibited poor clinical condition (including coldness, paleness, decreased motor activity, hunched posture, unkempt coat, eyelids partially closed, irregular/labored respiration), body weight loss and/or decreased food intake. Histopathologically, marked right and left ventricular and atrial dilatation of the heart with cardiac hemorrhage, cardiac inflammatory infiltrate, or cardiac cartilaginous metaplasia (chorda tendinea) were noted. The major factor contributing to death of all unscheduled decedents was cardiac toxicity resulting in heart failure. Additionally, alveolar and interstitial edema and congestion of lungs, liver congestion with centrilobular necrosis were noted in decedents. As a result, the top 2 doses were lowered from 2 to 1.2 and 1 to 0.6 mg/kg/day from day 12 to 15. In animals survived until scheduled necropsy marked and statistically significant increases of NT-proANP levels relative to control and pretest values were noted at 1/0.6 and 2/1.2 mg/kg/day. Ventricular dilatation, inflammatory- cell infiltrate/inflammation of the heart was present at all dose levels with a dose-dependency in incidence and severity. Mild ventricular dilatation was still present in the recovery animals. Based on histopathologic findings, a NOAEL could not be established.

Purpose

The primary objective of the study was to evaluate the toxicity, toxicokinetic profile and no observed adverse effect level of MYK-461 when administered orally to rats, once daily, for 13 weeks, and to estimate the potential for reversal of any toxic effects within a 4-week drug-free period.

Methods

Formulation

Test substance was suspended in 0.5% methylcellulose in distilled water. Drug formulations were prepared on varying days/weeks (days not specified) and refrigerated before use. Each of the prepared dosing formulations were evaluated for concentration. Analyses of the formulations demonstrated that MYK-461 in 0.5% Methylcellulose in distilled water was stable when stored at ambient temperature for 2 weeks.

Animals

Species/Strain: Rat, Sprague-Dawley Crl:CD(SD) from Charles River Labs
#/Sex/Group: 15 for the control and high dose groups (5 in each group were designated for the recovery phase), 10 for mid and low dose groups; and 6 (3 for the control) for the toxicokinetics study (Table 56)
Age: 7 weeks old at start of dosing
Weight: Males: 257 to 267 gm; Females: 173 to 187 gm at the start of dosing

Husbandry: Animals were housed in groups of 2 or 3 per cage. Food and water were available *ad libitum* throughout the study period except during the blood collection period for clinical pathology and necropsy where food was withdrawn.

Dosing

Doses: Animals were randomly assigned to four dose groups. Both main study and TK groups received test substance at doses of 0.3, 1 or 2 mg/kg/day (Table 56) for a targeted duration of 13 weeks. As a result of mortality and adverse effects observed at top 2 doses, 2 and 1 mg/kg/day were lowered to 1.2 and 0.6 mg/kg/day after a 4-day or 2 –day dosing-free period, respectively, from day 15 (main study animals) or day 12 (toxicokinetic animals) for the remainder of the dosing period (see footnote to Table 54). The adjusted doses for the remaining duration of the study were 0.3, 0.6 and 1.2 mg/kg/day. Dose levels were based on the results of a previous 6-wk oral toxicity study in rats in which test substance was administered at doses of 0.3, 1 or 3 mg/kg/day. Treatment at 3 mg/kg/day caused unscheduled deaths as a result of myocardial injury and heart failure. Test substance related microscopic findings in the heart were noted in animals survived to the end of dosing. NOAEL in this study was 1 mg/kg/day.

Table 56. Study design and dose levels

Group	Dose levels (mg/kg/day)	Main study animals			Toxicokinetic animals		
		Number/ sex	Animal numbers		Number/ sex	Animal numbers	
			Male	Female		Male	Female
1	0 [a]	10 + 5 in recovery [b]	1 to 15	51 to 65	3	101 to 103	122 to 124
2	0.3	10	16 to 25	66 to 75	6	104 to 109	125 to 130
3	1/0.6 [c]	10	26 to 35	76 to 85	6	110 to 115	131 to 136
4	2/1.2 [d]	10 + 5 in recovery [b]	36 to 50	86 to 100	6	116 to 121	137 to 142

[a] Aqueous solution of 0.5% (w/w) methylcellulose (400 cPs).

[b] The last 5 surviving animals/sex were kept on recovery.

[c] Dose level of 0.6 mg/kg/day was administered from study Day 15 (main animals) or 12 (toxicokinetic animals) after a 2-day dosing-free period (dose level of 1 mg/kg/day administered from study Days 1 to 12 in main animals, and from study Days 1 to 9 in toxicokinetic animals).

[d] Dose level of 1.2 mg/kg/day was administered from study Day 15 (main animals) or 12 (toxicokinetic animals) after a 4-day dosing-free period (dose level of 2 mg/kg/day administered from study Days 1 to 10 in main animals and from study Days 1 to 7 in toxicokinetic animals).

Mode and Duration of Administration: Once daily orally by gavage for all groups for 13 weeks. Control animals received drug vehicle (5 ml/kg). Recovery group animals were observed for an additional 4 weeks after administration of the last dose.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for mortality and clinical signs.

Body Weights: All animals were recorded twice pretest. For main study animals, body weights were recorded twice weekly for the first 2 weeks, then once weekly during the drug phase; and on days 98, 105, 112 and 119 during the recovery phase. For TK animals, body weights were recorded at least once weekly during the dosing period.

Food Consumption: Recorded pretest and twice weekly for the first 2 weeks and then once weekly for the rest of dosing period; on days 98, 105, 112 and 119 during the recovery phase.

Ophthalmoscopy: Conducted on all animals pretest and on day 88 in control and high dose groups only. Based on the results of day 88, no examination was done on other groups and during recovery period.

Hematology[4] and Clinical Chemistry[5]: Blood samples were collected from surviving animals from the tail vein (pretest, days 43 or 57) or abdominal aorta (at necropsy) from all main study animals (10/sex/group) at the following intervals:

- Once in pretest period for biomarkers (Troponin I (cTnI), N-terminal proatrial natriuretic peptide (NT-proANP) and potential biomarkers);
- 4 hours after dosing on days 43 (biomarkers only, all animals from groups 1 and 2) or 57 (biomarkers only, all animals from groups 3 and 4);
- Days 92 to 95 [all parameters, before necropsy (hematology and clinical chemistry) or at necropsy (coagulation and biomarkers) for animals euthanized at the end of the 3-month dosing period];
- Day 92 (all parameters except coagulation and biomarkers for animals designated for the 4-week recovery period).
- Day 120 [all parameters, before necropsy (hematology and clinical chemistry) or at necropsy (coagulation and biomarkers) for animals euthanized at the end of the 4-week recovery period].

Animals were fasted overnight and anesthetized with isoflurane before blood sampling at necropsy.

Urinalysis[6]: Urine samples were collected overnight from main study fasted animals at the end of treatment period and from recovery group fasted animals at the end of the recovery period. Access to water was allowed.

Gross Pathology: A complete necropsy including macroscopic examination of abnormalities and collection of tissues from an extensive list (Table 57) was done

[4] erythrocytes, hematocrit, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, red cell distribution width, reticulocytes, thrombocytes, white blood cell, white blood cell differential, fibrinogen, activated partial thromboplastin time, prothrombin time

[5] ALT, AST, AP, gamma-glutamyl transferase, glutamate dehydrogenase, total bilirubin, total protein, albumin, globulin, albumin/globulin ratio, glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, cholesterol

[6] pH, specific gravity, volume, bilirubin, creatinine, urobilinogen, occult blood, glucose

on all main study and recovery group animals fasted overnight at study termination. Early decedent rats (both main study and toxicokinetics) were also examined for the cause of death or moribund condition. Tissues with macroscopic observations plus brain, heart, kidney, liver, and lungs were collected and preserved.

Table 57. Tissues/organs sampled for histopathological examination

Tissue collection	Organ weights	Microscopic examination
Tissues with macroscopic observations (including masses)		X
Adrenal gland (2)	X	X
Aorta (thoracic)		X
Bone: femur		X
Bone: sternum		X
Bone marrow: femur		X
Bone marrow: sternum		X
Brain	X	X
Epididymis (2) [a]	X	X
Esophagus		X
Eye (2) [a]		X
Harderian gland (2) [a]		X
Heart [i]	X	X
Intestine: duodenum		X
Intestine: jejunum		X
Intestine: ileum		X
Intestine: cecum		X
Intestine: colon		X
Intestine: rectum		X
Intestine: gut-associated lymphoid tissue [b]		X
Joint: femorotibial		X
Kidney (2)	X	X
Lacrimal gland (2) [a]		X
Larynx [c]		
Liver	X	X
Lung (with bronchus)	X	X
Lymph node: mesenteric		X
Lymph node: mandibular (2) [d]		X
Mammary gland		X
Nasal tissue [c] (skull/nasal cavity)		
Nerve: optic (2) [a, d]		X
Nerve: sciatic (2)		X
Ovary (2)	X [g]	X
Oviduct (2)		X

Tissues/organs sampled for histopathological examination (continued)

Tissue collection	Organ weights	Microscopic examination
Pancreas		X
Parathyroid gland (2) [d]		X
Pituitary gland	X	X
Prostate gland	X [f]	X
Salivary gland: mandibular (2)		X
Salivary gland: sublingual (2)		X
Salivary gland: parotid (2)		X
Seminal vesicle (2)	X [f]	X
Skeletal muscle: diaphragm		X
Skeletal muscle: quadriceps femoris (cranial thigh)		X
Skeletal muscle: soleus (2) [h]		X
Skeletal muscle: gastrocnemius (2) [h]		X
Skin: subcutis		X
Spinal cord (cervical, thoracic and lumbar)		X
Spleen	X	X
Stomach: glandular		X
Stomach: nonglandular		X
Testis (2) [a]	X	X
Thymus	X	X
Thyroid gland	X [e]	X
Trachea		X
Tongue		X
Ureter (2)		X
Uterus (body and horns)		X
Uterus: cervix		X
Urinary bladder		X
Vagina		X

[a] Fixed in Davidson's fixative, except epididymides and testes from rats Nos.29 and 45 fixed in neutral buffered formalin. This deviation from the study plan was not considered to have impacted the validity of the study results.

[b] Gut-associated lymphoid tissue included in section of ileum or jejunum

[c] Retained in fixative; not processed

[d] Only one side required on slide

[e] Weighed with parathyroid gland, after fixation

[f] Prostate gland and seminal vesicle gland weighed together

[g] Weighed with oviduct

[h] With the surrounding tissues from the posterior part of the leg

[i] Two sections of heart on 2 slides, except the hearts of premature decedent animals (Nos. 44, 91, 93, and 95). Two slides with one section only were prepared for these hearts. This deviation from the study plan was not considered to have impacted the validity of the study results.

(2) Both sides sampled for paired organs

Organs Weighed: The terminal body weights and weights of the organ specified in Table 57 were recorded for all surviving main study and recovery group animals at the scheduled necropsy.

Histopathology: Microscopic examination was performed on all tissues listed in Table 57 from all main study animals in the control and the high dose groups sacrificed at the end of treatment and recovery periods and all prematurely

decedent animals. Additionally, all gross lesions and target organs (heart, liver and lung) were examined for all dose group main study animals sacrificed at termination.

Toxicokinetics: Blood samples for test substance determination were collected prior to dosing (on day 12), and 0.5, 1, 2, 4, 8 (6 h sampling instead of 4 and 8 h on day 12), 8 and 24 h after dosing on study days 1, 12, 42, 53, 88 and 91 from the tail vein or abdominal aorta (at necropsy) from all non-fasted, unanesthetized TK animals (3 rats/sex/time point) (Table 58). All TK animals except those scheduled for 24 h timepoint were euthanized and the carcasses were discarded without examination after blood collection. A necropsy was performed on animals scheduled for 24 h timepoint. One piece of left ventricle, soleus and diaphragm were collected from each animal at necropsy for test substance determination. Blood samples from control animals were collected at two time points (1 and 24 h post dose) only.

Table 58. Toxicokinetic blood sample collection timepoints and animal numbers

Group and sex		Day	Animal number by timepoint							
			Predose	0.5 h	1 h	2 h	4 h	8 h	24 h	30 h [d]
1 [a]	Males	1, 42 and 91	-	-	101 to 103	-	-	-	101 to 103	101 to 103
	Females		-	-	122 to 124	-	-	-	122 to 124	122 to 124
2	Males	1, 42 and 91	-	104 to 106	107 [e], 108, and 109	104 to 106	107 [e], 108, and 109	104 to 106	106 [g], 107 [e], 108, and 109	106, 108, and 109
	Females		-	125 to 127	128 to 130	125 to 127	128, 129, and 130 [e]	125 to 127	125 [g], 128, 129 and 130 [e]	125, 128 and 129
3	Males	1, 12, 53, and 88	113 to 115 [b]	110 to 112	113, 114 [e] and 115	110 to 112	113, 114 [e] and 115 [b]	110 to 112 [c]	112 [f], 113, 114 [e], and 115	112, 113, and 115
	Females		134 to 136 [b]	131 to 133	134 to 136	131 to 133	134 to 136 [b]	131 to 133 [c]	134 to 136	134 to 136
4	Males	1, 12, 53, and 88	119 to 121 [b]	116 to 118	119 to 121	116 to 118	119 to 121 [b]	116 to 118 [c]	119 to 121	119 to 121
	Females		140 to 142 [b]	137 to 139	140 to 142	137 to 139	140 to 142 [b]	137 to 139 [c]	140 to 142	140 to 142

[a] Aqueous solution of 0.5% (w/w) methylcellulose (400 cPs).

[b] On Day 12, this subset of animals was sampled before dosing instead of 4 h after dosing.

[c] On Day 12, this subset of animals was sampled 6 h after dosing instead of 8 hours after dosing.

[d] This subset of animals was sampled at necropsy (ie, approximately 30 hours after dosing on Day 88 or 91; see Section 6.14.2.1)

[e] Due to mortality, animals Nos.107 and 114 were not sampled on Day 91 or 88, respectively. Animal No. 130 was not sampled at 4 and 24 hours after dosing on Day 91.

[f] On Day 88, animal No.112 was also sampled 24 hours after dosing.

[g] On Day 91, animals Nos.106 and 125 were also sampled 24 hours after dosing.

Results

Analysis of Formulations: The achieved concentrations of MYK-461 for all doses were in the range from 80 to 120% of nominal.

Mortality: A male (#44) and two females (#91, 95) receiving 2 mg/kg/day were euthanized or found dead on study day 10 (Table 59). Prior to death or euthanasia, clinical signs consisted of coldness, paleness, decreased motor activity, hunched posture, unkempt coat, eyelids partially closed, irregular or labored respiration, body weight loss and decreased food intake. The cause of death was heart failure. Histopathologically, marked left and right ventricular and atrial dilatation of the heart associated with mild to marked alveolar and interstitial edema and moderate congestion of lungs, mild to moderate liver congestion with mild to marked centrilobular necrosis were noted in these animals. The marked heart dilatation in a male rat (#44) was associated with a "large" heart recorded at necropsy. Although there were no test substance-related deaths at 1 mg/kg/day, the sponsor decided to lower the dose of 2 mg/kg/day to 1.2 and 1 mg/kg/day to 0.6 mg/kg/day from day 15 for main study and day 12 for toxicokinetic animals onwards after a 4- or 2-day dosing free period, respectively. No test substance-related deaths occurred for the remainder period of the study at any of the doses.

Table 59. Mortality at 2 mg/kg/day

Dose (mg/kg/day)	Animal number (Sex)	Day of death	Death status	In-life findings	Main pathology findings
2	44 (M)	10	FD	-	Heart enlarged macroscopically; marked left and right ventricular dilatation; marked left and right atrial/auricular dilatation; moderate congestion and mild edema of lungs; marked centrilobular necrosis and moderate congestion of liver
	91 (F)	10	HE	Day 10: Emaciated aspect, hyporeactivity, decreased motor activity, labored respiration, increased respiration rate, paleness, partially closed eyelids Body weight loss (Days 7-10): -5%	Marked left ventricular dilatation; marked left and right atrial/auricular dilatation; moderate edema and mild congestion of lungs; mild centrilobular necrosis and congestion of liver
	93 (F)	10	HE	Day 10: Emaciated aspect, hyporeactivity, decreased motor activity, labored respiration, paleness, closed eyelid(s), unkempt coat, hunched posture, partially closed eyelids Body weight loss (Days 7-10): -5%	Marked left and right ventricular dilatation; moderate left and right atrial/auricular dilatation; marked edema and mild congestion of lungs
	95 (F)	10	HE	Day 9 and/or 10: Emaciated aspect, hyporeactivity, decreased motor activity, labored/irregular respiration, increased respiration rate, paleness, unkempt coat, hunched posture, cold to touch Body weight loss (Days 7-10): -12%	Marked left and right ventricular dilatation; moderate left and right atrial/auricular dilatation; marked edema and mild congestion of lungs; marked centrilobular necrosis and mild congestion of liver

FD: found dead; HE: humane euthanasia

There were three early decedents in the TK group animals that were caused by gavage error. Two males one each receiving 0.3 (#107) and 1/0.6 (#114)

mg/kg/day were euthanized on days 61 and 74, respectively. A female (#130) receiving 0.3 mg/kg/day was found dead on day 91. Both macroscopic and microscopic observations suggested hemorrhages, necrosis, inflammation and red foci in lungs of these animals.

Clinical Signs: No test substance-related clinical signs in surviving animals after reducing the top two dose levels.

Body Weights: A non-dose-dependent decrease in body weight gain relative to control was noted (higher in males than in females) at all dose levels for the study period between days 1 and 14. A similar trend was noted after the dose levels were adjusted on day 15, i.e., from day 14 to day 91 (Table 60). However, the sponsor considers these changes as not statistically significant and is an expected biological variation.

Table 60. Group mean body weight gain and percent change in mean body weight in rats dosed with MYK-461 for 13 weeks

Dose (mg/kg/day)	MALES					FEMALES				
	Body weight gain		Body weight [a]			Body weight gain		Body weight [a]		
	Days 1-14	Days 14-91	Day 1	Day 14	Day 91	Days 1-14	Days 14-91	Day 1	Day 14	Day 91
0	100	234	-	-	-	38	99	-	-	-
0.3	84 (-16)	204 (-13)	+2	-3	-7	39 (+3)	92 (-7)	-7	-6	-6
1/0.6 [b]	76 (-24)	218 (-7)	-1	-7	-7	34 (-11)	88 (-11)	-5	-6	-8
2/1.2 [c]	88 (-12)	198 (-15)	-4	-6	-10	34 (-11)	93 (-6)	-2	-4	-4

Body weight gains in grams (percent of changes compared to control group).

Mean Body weight: Percent changes versus control group.

[a] Surviving animals (ie, excluding decedents animals).

[b] Dose level of 1 mg/kg/day was administered from study Days 1 to 12. Dose level of 0.6 mg/kg/day was administered from study Day 15 after a 2-day treatment-free period.

[c] Dose level of 2 mg/kg/day was administered from study Days 1 to 10. Dose level of 1.2 mg/kg/day was administered from study Day 15 after a 4-day treatment-free period.

Food Consumption: No test substance-related effects on food consumption.

Ophthalmoscopy: No significant changes

Hematology: No test substance-related effects.

Clinical Chemistry: No test substance-related effects in clinical chemistry parameters. Regarding biomarkers, marked and statistically significant increases of NT-proANP relative to control and pretest values were noted at 2/1.2 mg/kg/day in males at Week 6 (Table 61) and in females at Week 6 and end of dosing as well as at 1/0.6 mg/kg/day at Week 6 (Table 61). Although microscopic ventricular dilatation with or without an increase in heart weight relative to control was noted in most of the rats at all dose levels, only 3 female rats (#74, #81 and #90 at 0.3, 1/0.6 and 2/1.2 mg/kg/day, respectively) had increased NT-proANP

levels above the range of control values. The reason for poor correlation is attributed to considerable variation in NT-proANP individual values. Troponin I levels were slightly higher in a few high-dose rats from both sexes than were in control at the end of the 4-week recovery period. However, a compound-relationship remained doubtful because of the lack of supportive evidence of myocardial necrosis.

Table 61. Group mean NT-proANP levels in rats receiving MYK-461 for 13 weeks

MALES

Study Period	0 mg/kg/day		0.3 mg/kg/day		1/0.6 mg/kg/day [a]		2/1.2 mg/kg/day [b]	
	NT-proANP (mean)		NT-proANP (mean)		NT-proANP (mean)		NT-proANP (mean)	
	nmol/L	% changes from pretest	nmol/L (% of controls)	% changes from pretest	nmol/L (% of controls)	% changes from pretest	nmol/L (% of controls)	% changes from pretest
Week 6 [c]	0.997	-7	0.931 (-7)	+1	1.028 (+3)	+8	1.533 (+54)	**+46**
Week 11/13 [d]	0.896	-8	0.984 (+10)	+1	0.866 (-3)	-1	1.034 (+15)	+7
Recovery	0.908	-25	-	-	-	-	0.921 (+1)	-19

FEMALES

Study Period	0 mg/kg/day		0.3 mg/kg/day		1/0.6 mg/kg/day [a]		2/1.2 mg/kg/day [b]	
	NT-proANP (mean)		NT-proANP (mean)		NT-proANP (mean)		NT-proANP (mean)	
	nmol/L	% changes from pretest	nmol/L (% of controls)	% changes from pretest	nmol/L (% of controls)	% changes from pretest	nmol/L (% of controls)	% changes from pretest
Week 6 [c]	0.594	-14	1.078 (+81)	-21	1.678 (+182)	**+234**	1.433 (+141)	**+306**
Week 11/13 [d]	0.800	+32	0.799 (0)	**-37**	0.897 (+12)	+68	0.885 (+11)	**+195**
Recovery	0.558	-40	-	-	-	-	0.608 (+9)	-17

In bold: statistically significant when compared to controls. Statistical analysis was performed on the log-transformed ratio from pretest.

[a] Dose level of 1 mg/kg/day was administered from study Days 1 to 12. Dose level of 0.6 mg/kg/day was administered from study Day 15 after a 2-day treatment-free period.

[b] Dose level of 2 mg/kg/day was administered from study Days 1 to 10. Dose level of 1.2 mg/kg/day was administered from study Day 15 after a 4-day treatment-free period.

[c] Week 6 corresponds to Day 43 for dose level of 0.3 mg/kg/day, or Day 57 for the adjusted dose levels of 0.6 and 1.2 mg/kg/day.

[d] Week 13 corresponds to the end of the dosing period for dose level of 0.3 mg/kg/day; Week 11 corresponds to the end of the dosing period of the adjusted dose levels of 0.6 and 1.2 mg/kg/day.

Urine analysis: No effect.

Gross Pathology: A 'large' heart was noted for a male (#40, Table 62) and a female (#89, Table 63) receiving the high dose. For the male, it correlated with the microscopic finding of moderate ventricular dilatation, the most severe grade of dilatation among rats euthanized as scheduled.

Table 62. Heart - Comparison of microscopy, macroscopic observations, organ weights and NT-proANP-levels in male rats receiving MYK-461 for 13 weeks

Sex	Animal No.	Dose (mg/kg/day)	Microscopy	Macroscopic observation	Increase in relative heart weights compared to upper limit of range of controls in %	Increased NT-proANP values in nmol/L at Week 6 when compared to upper limit of range of control and pre-test values
Males	17	0.3	-	-	+26	-
	20		Dilatation: left ventricle, minimal	-	-	-
	24		Dilatation: left ventricle, minimal	-	-	-
	25		-	-	+53	-
	26	1/0.6	Dilatation: left ventricle, minimal	-	-	-
	28		**Dilatation: left ventricle, minimal**	-	**+16**	-
	29		-	-	+11	-
	30		Dilatation: right ventricle, mild	-	-	-
	36	2/1.2	Dilatation: right ventricle, minimal	-	-	-
	37		Dilatation, left and right ventricle, minimal	-	-	-
	39		-	-	+13	-
	40		**Dilatation: left ventricle and atrium/ auricle, moderate**	**Heart: large**	**+64**	-
	43		-	-	+24	-
	45		-	-	+29	-

Table 63. Heart - Comparison of microscopy, macroscopic observations, organ weights and NT-proANP-levels in female rats receiving MYK-461 for 13 weeks

Sex	Animal No.	Dose (mg/kg/day)	Microscopy	Macroscopic observation	Increase in relative heart weights compared to upper limit of range of controls in %	Increased NT-proANP values in nmol/L at Week 6 when compared to upper limit of range of control and pre-test values
Females	69	0.3	-	-	+31	-
	70		Dilatation: right ventricle, mild	-	-	-
	72		-	-	+18	-
	73		-	-	+11	-
	74		**Dilatation: right ventricle, mild**	**-**	**-**	**2.683**
	80	1/0.6	Dilatation: right ventricle, mild	-	-	-
	81		-	-	-	4.096
	83		-	-	+21	-
	84		-	-	+16	-
	85		-	-	+11	-
	86	2/1.2	-	-	+14	-
	88		-	-	+47	-
	89		-	Heart: large	+17	-
	90		**Dilatation: left and right ventricle, mild**	**-**	**+53**	**2.496**
	92		-	-	+18	-
	94		**Dilatation: left and right ventricle, minimal**	**-**	**+10**	-

"-": no compound related changes / no findings

Organ Weights: Treatment-related minimal to moderately and dose-dependently increase in heart weight (absolute and relative to body weights) relative to control was noted (up to 33% in males or 39% in females) at all doses but reached statistically significance for high dose groups only (Table 64). However, it poorly correlated with ventricular dilatation at the individual level as only 4/20 rats with heart weights above the control group range had microscopic dilatation. An increase ($P < 0.05$) in mean absolute and relative heart weights was still noted for the high dose group after the 4-week recovery period (Table 65).

Table 64. Group mean organ weights (absolute and relative to body weight) in rats dosed with MYK-461 for 13 weeks (terminally sacrificed)

MALES

ORGAN	DOSE GROUP: / NO.ANIMALS:	1 10	2 10	3 10	4 10
HEART	n:	10	10	10	9
	MEAN WEIGHT (g):	1.71	1.86	1.91	2.10*
	SD :	0.188	0.338	0.274	0.400
	DEVIAT.FROM CONTR.(%):	—	8.55	11.34	22.56
	MEAN % BODY :	0.31322	0.35968	0.36790	0.41633**
	SD :	0.041	0.091	0.034	0.090
	DEVIAT.FROM CONTR.(%):	—	14.84	17.46	32.92

FEMALES

HEART	n:	10	10	10	7
	MEAN WEIGHT (g):	1.14	1.22	1.16	1.46**
	SD :	0.142	0.158	0.228	0.206
	DEVIAT.FROM CONTR.(%):	—	7.21	1.41	28.49
	MEAN % BODY :	0.37309	0.43772*	0.41807	0.51928**
	SD :	0.030	0.061	0.057	0.079
	DEVIAT.FROM CONTR.(%):	—	17.32	12.06	39.18

Dose groups: 1, 2, 3, 4 are respectively, 0, 0.3, 1/0.6, 2/1.2 mg/kg/day.

Table 65. Group mean organ weights (% difference relative to controls) in rats dosed with MYK-461 for 13 weeks and following 4-week recovery phase

Sex		Male	Female
Dose (mg/kg/day)		**2/1.2**	**2/1.2**
Change in Terminal Body Weight		**-15**	**-1**
Heart	Absolute	+14	+20 *
	Relative	+33 *	+22 *

"-": No compound-related changes; * statistically significant by Dunnet's test

Histopathology: Test substance-related findings were noted exclusively in the heart at all dose levels. All 4 animals sacrificed prematurely (3 females and 1 male) in the high dose group (see mortality Table 59), had moderate to marked ventricular and atrial dilatation with cardiac hemorrhage (1 female), cardiac

inflammatory infiltrate (another female), or cardiac cartilaginous metaplasia (Chorda tendinea, 2 females). Furthermore, these animals were associated with edema and congestion of lungs with hemorrhage as well as congestion of the liver with centrilobular necrosis. In terminal sacrificed animals, minimal to moderate ventricular dilatation, inflammatory- cell infiltrate/inflammation was present at all dose levels with a dose-dependency in incidence and severity. Left and right ventricular dilatation together was more prevalent at the high dose level (Table 66). Myocardial fibrosis was noted in a few males at all dose levels. These findings were associated with a statistically significantly increased absolute and relative heart weights in the high dose group. Moderate atrial dilatation was observed in a high dose male rat. The findings were observed at left, right or left and right heart. After the 4-week recovery period, minimal to mild ventricular dilatation was still present in 1/5 males and 3/5 females (Table 67). Based on histopathological findings in the heart noted at all dose levels, a NOAEL could not be established.

Table 66. Test article-related microscopic findings in the heart in rats dosed with MYK-461 for 13 weeks (scheduled necropsy)

Sex		Males			Females		
	Dose (mg/kg/day)	0.3	1/0.6	2/1.2	0.3	1/0.6	2/1.2
	Number of animals	10	10	9	10	10	7
HEART							
Dilatation: ventricle (general diagnosis without localization)							
	Minimal	2	2	2	-	-	1
	Mild	-	1	-	2	1	1
	Moderate	-	-	1	-	-	-
Dilatation: ventricle, left and right							
	Minimal	-	-	1	-	-	1
	Mild	-	-	-	-	-	1
Dilatation: ventricle, left							
	Minimal	2	2	-	-	-	-
	Mild	-	-	-	-	-	-
	Moderate	-	-	1	-	-	-
Dilatation: ventricle, right							
	Minimal	-	-	1	-	-	-
	Mild	-	1	-	2	1	-
Dilatation: atrium/auricle, left							
	Moderate	-	-	1	-	-	-

"-": no compound related findings

Table 67. Test article-related microscopic findings in the heart in rats dosed with MYK-461 for 13 weeks and following 4-week necropsy phase

Sex	Males	Females
Dose (mg/kg/day)	2/1.2	2/1.2
Number of animals	5	5
HEART		
Dilatation: ventricle (general diagnosis without localization)		
Minimal	1	1
Mild	-	2
Dilatation: ventricle, left and right		
Mild	-	2
Dilatation: ventricle, left		
Minimal	1	1

"-": no compound related findings

Toxicokinetics: As noted previously under Observations and Measurements, blood was sampled for determination of MYK-461 on study days 1, 12, 42, 53, 88 and 91 following oral administration of MYK-461 at dose levels 0.3, 1/0.6 and 2/1.2 mg/kg/day. Peak concentration was between 0.4 and 1 h post dosing except on day 1 at 0.3 mg/kg/day where the Tmax was at 2 h post dosing. Systemic exposures (Cmax and AUC) increased approximately proportionately to the increase in dose on all days of measurement. Although there was no sex-dependent difference, the systemic exposure of female rats to MYK-461 was generally higher than that of males. Accumulation (in the range of 1.3 to 2.9) was noted after repeated daily oral doses. The data suggest that the steady state was reached after 42 days of treatment (Table 68).

MYK-461 was largely distributed in tissues (left ventricle, soleus and diaphragm) collected from each animal, 30 h after dosing at necropsy. Individual tissue to plasma concentration ratios ranged from:
left cardiac ventricle: 4 to 13, 12 to 21 and 9 to 17,
soleus muscle: 2 to 13, 3 to 7 and 2 to 13,
diaphragm: 2 to 5, 3 to 8 and 4 to 5, respectively, at 0.3, 1/0.6 and 1/1.2 mg/kg/day.

Table 68. Summary of MYK-461 exposure data in 13-week oral study in rats

Sex	Dose level (mg/kg/day)	Day	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24} (ng.h/mL)
Male	0.3	1	43.2	2.0	650
		42	127	0.50	1780
		91	180	1.0	3060
Male	0.6	12	180	0.50	2060
		53	316	1.0	3220
		88	357	0.50	4260
Male	1.0	1	147	1.0	2150
Male	1.2	12	288	1.0	3360
		53	602	0.50	7910
		88	463	1.0	6730
Male	2.0	1	292	4.0	4550
Female	0.3	1	69.7	0.50	784
		42	152	0.50	1820
		91	145	0.40	2170
Female	0.6	12	233	1.0	3310
		53	303	0.50	4670
		88	291	0.50	4770
Female	1.0	1	295	0.50	4650
Female	1.2	12	464	0.50	6330
		53	923	0.50	15900
		88	719	1.0	13000
Female	2.0	1	454	0.50	7060

Dose level of 0.3 mg/kg/day was administered from study Day 1.
Dose level of 0.6 mg/kg/day was administered from study Day 12 (toxicokinetic animals) after a 2-day dosing-free period (dose level of 1 mg/kg/day was administered from study Days 1 to 9 in toxicokinetic animals). Accordingly, Day 12 corresponds to the Day 1 of the adjusted dose level.
Dose level of 1.2 mg/kg/day was administered from study Day 12 (toxicokinetic animals) after a 4-day dosing-free period (dose level of 2 mg/kg/day was administered from study Days 1 to 7 in toxicokinetic animals). Accordingly, Day 12 corresponds to the Day 1 of the adjusted dose level.
AUC_{0-24}: Area under the plasma concentration time curve calculated by the linear trapezoidal method from time 0 to 24 h post dose.
Cmax: Maximum observed concentration; tmax: Time of maximum observed concentration

6.2.5 26-Week oral toxicity study in rats followed by a 3-month recovery period

Conducting laboratory and location: (b) (4)

Sponsor study/report number: NC-16-0049
Testing facility study number: 8334141
Date of study initiation: December 11, 2015
Date of last necropsy: September 16, 2016
Drug, Lot number: MYK-461, 15B0013 ((b) (4) lot 150030), 99.98% purity
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

Daily oral administration of 1.2 mg MYK-461/kg resulted in death of a male and a female on study days 95 and 142, respectively. These animals showed no prior abnormal clinical signs and cause of death was cardiac toxicity resulting in heart failure. Echocardiogram evaluation in surviving high dose animals showed an extensive increase in heart size and a reduction in systolic function as indicated by an increase in LV end diastolic volume and systolic volume, and reduction in left ventricular systolic performance that correlated to cardiac dilation. LVEDVs tended to be higher (with no correlates to cardiac dilation) than control in rats receiving 0.6 mg/kg/day. These effects were reversible by week 10 of the recovery phase. Histopathologic examination of the heart showed presence of osseous/cartilaginous metaplasia in males receiving 1.2 mg/kg/day and females receiving ≥0.6 mg/kg/day. This was considered a permanent change and non-reversible. Based on the histopathological findings in the heart noted, the NOAEL was 0.3 mg/kg/day.

Purpose

The primary objective of the study was to evaluate the toxicity, toxicokinetic profile and no observed adverse effect level of MYK-461 when administered orally to rats, once daily, for 26 weeks, and to estimate the potential for reversal of any toxic effects within a 3-month drug-free period.

Methods

Formulation

Test substance was suspended in 0.5% methylcellulose in distilled water. Drug formulations were prepared weekly and refrigerated before use. Formulations prepared during weeks 1, 5, 7, 13 and 26 were evaluated for concentration. Analyses of the formulations demonstrated that MYK-461 in 0.5% methylcellulose in distilled water was stable when stored at ambient temperature for 2 weeks.

Animals

Species/Strain: Rat, Sprague-Dawley Crl:CD(SD) from Charles River Labs
#/Sex/Group: 15 for the control and high dose groups (5 in each group were designated for the recovery phase), 10 for mid and low dose groups; and 6 (3 for the control) for the toxicokinetics study (Table 69)
Age: 7 weeks old at start of dosing
Weight: Males: 161 to 230 gm; Females: 146 to 222 gm at the start of dosing
Husbandry: Animals were housed in groups of 2 or 3 per cage. Food and water were available *ad libitum* throughout the study period except during the blood collection periods for clinical pathology and necropsy where food was withdrawn.

Dosing

Dose and Duration of Administration: Animals were randomly assigned to four dose groups. Both main study and TK groups received test substance at doses of 0.3, 0.6 or 1.2 mg/kg/day (Table 69) for up to 182 days at a volume of 5 ml/kg. Recovery group (1 and 4) animals were observed for an additional 3 months after administration of the last dose.

Table 69. Study design and dose levels

Group[a]	Subgroup[b]	No. of Animals		Dose Level	Dose Concentration
		Male	Female	(mg/kg/day)	(mg/mL)
1 (Control)	1 (Toxicity)	15	15	0	0
	2 (Toxicokinetic)	3	3	0	0
2 (Low)	1 (Toxicity)	10	10	0.30	0.06
	2 (Toxicokinetic)	6	6	0.30	0.06
3 (Mid)	1 (Toxicity)	10	10	0.6	0.12
	2 (Toxicokinetic)	6	6	0.6	0.12
4 (High)	1 (Toxicity)	15	15	1.2	0.24
	2 (Toxicokinetic)	6	6	1.2	0.24

a Group 1 was administered vehicle control article only.
b Toxicity animals: terminal euthanasia of 10 animals/sex/group (dependent on survival) after at least 26 weeks of dosing. Animals designated for recovery euthanasia (five animals/sex from Groups 1 and 4) underwent at least 3 months of recovery following the dosing phase. All surviving toxicokinetic animals were euthanized and discarded after the last blood collection.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for mortality and clinical signs.
Body Weights: All animals were recorded once pretest. For main study animals, body weights were recorded before dosing on day 1, weekly for the first 26 weeks, then on day 182 of the dosing phase; and weekly up to week 13 and on day 91 of the recovery phase. For TK animals, body weights were recorded once weekly up to week 26 of the dosing phase.

Food Consumption: Recorded pretest and weekly to week 26, from days 176 to 182 of the dosing phase; weekly to week 13 and days 85 to 91 of the recovery phase.

Ophthalmoscopy: Conducted on all animals pretest and once for all toxicity group animals on day 179 of the dosing phase.

Hematology[1] and Clinical Chemistry[2]: Blood samples were collected from surviving main study fasted animals from the jugular vein once during the week 13 of the dosing phase and on days of scheduled euthanasia. Blood samples were also collected for NT-proBNP analysis from main study non-fasted animals from the jugular vein once on day 1, during week 13 of the dosing phase, and on days of scheduled euthanasia.

Urinalysis[3]: Urine samples were collected overnight from main study fasted animals once during the week 13 of the dosing phase and on days of scheduled euthanasia.

Echocardiogram Examinations: Echocardiograms were recorded from anesthetized main study animals once during week 25 of the dosing phase, and during weeks 4 and 10 of the recovery phase.

Toxicokinetics: Blood samples for test substance determination were collected on days 1, 91 and 26 of the dosing phase from the jugular vein from all non-fasted TK animals (Table 70). Blood was also collected from animals euthanized at an unscheduled interval. All TK animals were euthanized, and the carcasses were discarded without examination after blood collection.

Table 70. Toxicokinetic blood sample collection timepoints

Group	Set	Dosing Phase Day	Time Points[a]
Group 1	All animals	Day 1, Week 13, Week 26	2 hours
Groups 2-4	1st three/sex/group	Day 1, Week 13[b] and Week 26[b]	1, 4, and 24 hours postdose
Groups 2-4	2nd three/sex/group	Day 1, Week 13[b] and Week 26[b]	0.5, 2, and 8 hours postdose

Note: When an animal assigned to a specific time point died or was euthanized prior to its scheduled sample collection, another animal in the same dose group was used.

a Blood collection times were approximate.

b Collections in Weeks 13 and 26 of the dosing phase were done on Days 91 and 182, respectively.

[1] erythrocytes, hematocrit, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, reticulocytes, thrombocytes, white blood cell, white blood cell differential, activated partial thromboplastin time, prothrombin time

[2] ALT, AST, AP, gamma-glutamyl transferase, total bilirubin, total protein, albumin, globulin, albumin/globulin ratio, glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, cholesterol

[3] pH, specific gravity, volume, bilirubin, urobilinogen, occult blood, glucose, ketones

Gross Pathology: A complete necropsy including macroscopic examination of abnormalities and collection of tissues from an extensive list (Table 71) was done on all main study and recovery group animals fasted overnight at study termination. Early decedent rats (both main study and toxicokinetics) were also examined for the cause of death or moribund condition.

Table 71. Tissues/organs sampled for histopathologic examination

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	muscle, biceps femoris		P,E
animal identification chip[a]			optic nerve (2)[b]		P,E
aorta		P,E	ovary (2)	W	P,E
brain	W	P,E	pancreas		P,E
cecum		P,E	pituitary gland	W	P,E
cervix		P,E	prostate	W	P,E
colon		P,E	rectum		P,E
duodenum		P,E	salivary gland (mandibular [2])	W	P,E
epididymis (2)	W	P,E	sciatic nerve		P,E
esophagus		P,E	seminal vesicle	W	P,E
eye (2)[b]		P,E	skin/subcutis		P,E
femur with bone marrow (articular surface of the distal end)		P,E	spinal cord (cervical, thoracic, and lumbar)		P,E
Harderian gland[b]		P,E	spleen	W	P,E
heart[c]	W	P,E	sternum with bone marrow		P,E
ileum		P,E	stomach		P,E
jejunum		P,E	testis (2)[b]	W	P,E
kidney (2)	W	P,E	thymus	W	P,E
lesions		P,E	thyroid (2 lobes) with parathyroid	W	P,E
liver	W	P,E	tongue		P,E
lungs with large bronchi	W	P,E	trachea		P,E
lymph nodes (mandibular)		P,E	urinary bladder		P,E
lymph nodes (mesenteric)		P,E	uterus	W	P,E
mammary glands (females)		P,E	vagina		P,E

E = Examined microscopically; P = Processed; W = Weighed.

a Retained but not processed for routine microscopic examination.

b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

c For scheduled and unscheduled euthanasias, the heart was stored in cold 10% neutral-buffered formalin after weighing. Tissues were transferred to a refrigerator, set to maintain 2 to 8°C, until processed. Heart tissue for animals found dead were collected and stored in 10% neutral-buffered formalin under ambient conditions.

Organs Weighed: The terminal body weights and weights of the organ specified in Table 71 were recorded for all surviving main study and recovery group animals at scheduled necropsy.

Histopathology: Microscopic examination was performed on all tissues listed in Table 71 from all main study animals in the control and the high dose groups sacrificed at the end of treatment and recovery periods and all prematurely decedent animals. Additionally, all gross lesions and target organs (heart and liver) were examined for all dose group main study animals sacrificed at termination.

Results

Analysis of Formulations: The achieved concentrations of MYK-461 for all formulations prepared on day 1 and week 5 were out of specification and were in the range from 76.4 to 87.8% of nominal concentrations. However, formulations for weeks 7, 13, and 26 were within specification (±10% of nominal concentration).

Mortality: Five animals, three from main study and two from toxicokinetic groups died during the study. A toxicokinetic male (#B52455) receiving 1.2 mg/kg/day was found dead on study day 95. No necropsy was carried out and was considered related to administration of test substance. A main study female rat (#B52515) receiving 1.2 mg/kg/day was found dead on study day 142 without prior abnormal clinical signs. Macroscopic findings included an enlarged heart and dark red abdominal fluid. MYK-461-related microscopic findings correlated to the enlarged heart were moderate cardiac left ventricle and slight cardiac right ventricle dilatation. Moderate centrilobular degeneration and congestion of the liver and moderate diffuse pulmonary alveolar macrophage infiltrates, sometimes having pigment were noted. Adrenal cortex had moderate congestion/ hemorrhage and slight necrosis. The thymus had moderate lymphocyte depletion/necrosis. Two main study males, one each from 0.6 (#B52427) and 0.3 (#B52408) mg/kg/day died on study days 89 and 22, respectively, were considered accidental. Additionally, a toxicokinetic female (#B52491) receiving 0.3 mg/kg/day was euthanized on study day 64 based on clinical observations of irregular respiration and clear oral discharge. It was suggested that the animal might have aspirated test substance during dosing.

Clinical Signs: No test substance-related clinical signs were demonstrated during the dosing or recovery phase.

Body Weights: A non-dose-dependent sporadic changes in body weight gain were observed and were considered incidental.

Food consumption: Test substance did not affect mean food consumption during the dosing or recovery phase.

Ophthalmoscopy: No significant changes

Hematology: A minimal increase in RBC, hemoglobin, and hematocrit (≤+7%) on day 183 in females at all dose levels, and platelet count (≤+15%) on days 89 and 183 of the dosing phase in high dose males was noted. Reversibility was observed except for the platelet count. Given the small magnitude of change and only in one sex, the sponsor does not consider these findings toxicologically important adverse effect.

Clinical Chemistry: Moderate increases (≥2.0 to 13.5 times of individual value compared to concurrent control mean value) in liver enzymes, ALT and AST, were noted non-dose-dependently in most of the animals receiving ≥0.6 mg/kg/day on study day 183. Evidence of returning to control values were noted in a few animals on day 92 of the recovery phase. According to the sponsor, the elevated enzyme levels were suggestive of hepatocellular damage. Histologic liver finding of focal/multifocal necrosis was seen in some terminal euthanized MYK-461-treated animals with or without liver enzymes changes. Transient minimal increases in NT-proBNP were noted in both control and treated groups on study days 89 and 183.

Urinalysis: Test substance had no effect on urinalysis parameters.

Echocardiogram Examinations: ECGs were not recorded. High dose group animals demonstrated during week 25 of the dosing phase an increase in LV end diastolic volume (~26%) and LV end systolic volume (~92%) with a reduction in global systolic performance (i.e., ~31% reduction in LV ejection fraction) relative to control animals. These effects were correlated by similar trends for diameter-based measures, which included statistically significant differences in increased mean left ventricle internal chamber diameter (end diastolic [LVIDd]) for females receiving 1.2 mg/kg/day and end-systolic [LVIDs] for males and females receiving 1.2 mg/kg/day) and decreased left ventricle fractional shortening (LV FS) for both sexes (Fig. 43). These animals also showed a reduction in stroke volume that correlated to cardiac dilation. LVEDVs tended to be higher (with no correlates to cardiac dilation) than control in rats receiving 0.6 mg/kg/day. These findings correlated with increased heart weight and histopathologic changes of osseous/ cartilaginous metaplasia of left ventricular trabeculae. An increase in heart rate was noted at all dose levels relative to control. A reversibility in these parameters including stroke volume and heart rate were comparable with control animals by week 10 of the recovery phase indicating complete recovery.

Gross Pathology: An unscheduled high dose female (#B52515) revealed a 'large' heart and dark red abdominal fluid. No other macroscopic findings were noted during the terminal or recovery phase euthanasias attributable to MYK-461.

Organ Weights: Treatment-related moderate and dose-dependent increase in heart weight (absolute and relative to body and brain weights) relative to control

was noted (up to 8% in males or 25% in females) at all doses but reached statistically significance for the high dose group females only (Table 72). At the recovery necropsy, the heart weight effects were reversed. Additionally, statistically significantly lower and higher thymus weights for males and females, respectively, relative to control occurred (Table 73) with no histopathologic correlates.

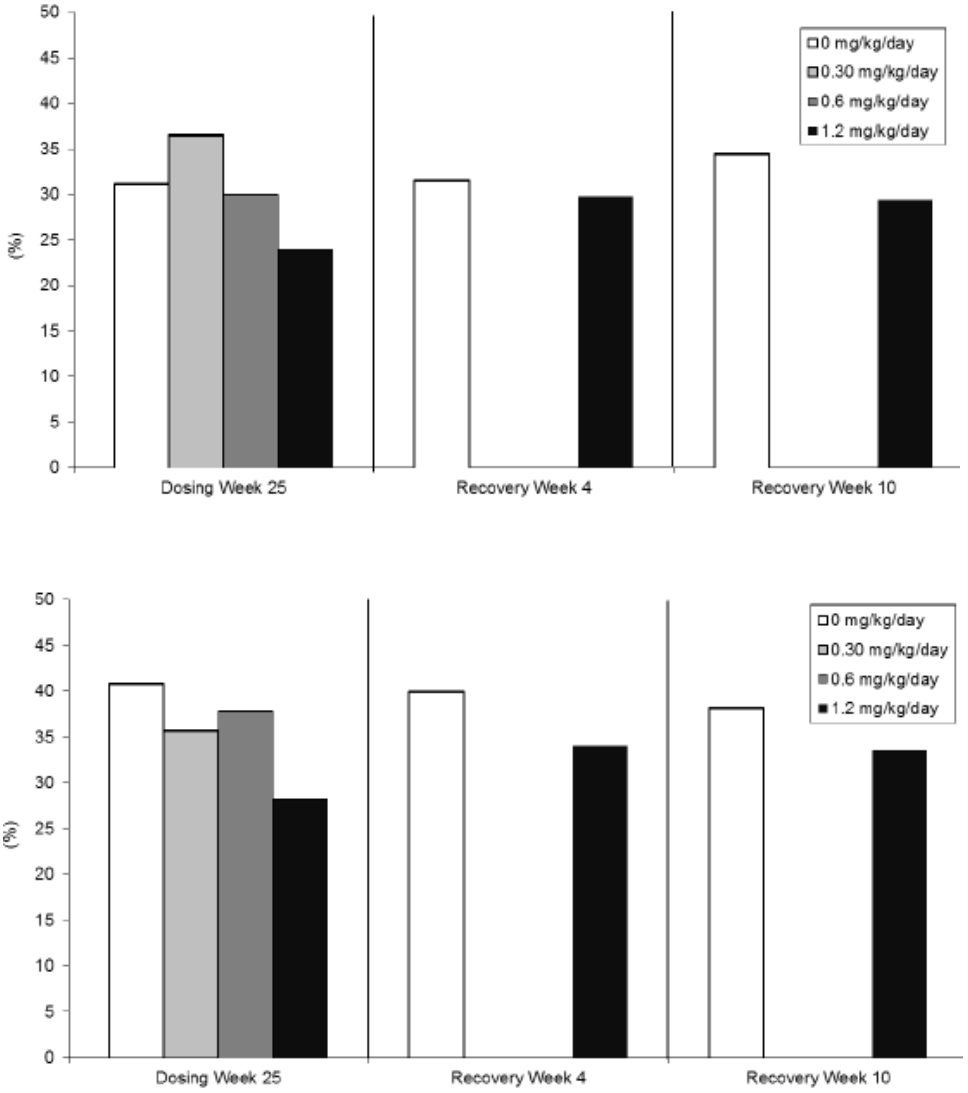


Figure 43. Left ventricular fractional shortening data - males (top) and females (bottom)

Table 72. Group mean organ weights (absolute and relative to body/brain weight) in rats dosed with MYK-461 for 26 weeks – Terminal euthanasia

		MYK-461						
Sex	Male				Female			
Dose Level (mg/kg/day)	0	0.30	0.6	1.2	0	0.30	0.6	1.2
Heart								
Absolute Weight (g)	1.5709	95	102	105	1.0702	101	104	118*
Body Weight Ratio (%)	0.2541	98	105	108	0.2961	106	111	125*
Brain Weight Ratio (%)	70.0062	94	102	105	51.0317	101	105	119*

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for MYK-461-treated groups expressed as percentage control mean value.

Table 73. Group mean organ weights (% difference relative to controls) in rats dosed with MYK-461 for 26 weeks and following 4-week recovery phase – Recovery euthanasia

		MYK-461			
Sex	Male		Female		
Dose Level (mg/kg/day)	0	1.2	0	1.2	
Heart					
Absolute Weight (g)	1.7211	92	1.1010	113	
Body Weight Ratio (%)	0.2507	99	0.3072	89	
Brain Weight Ratio (%)	74.8714	90	52.3555	113	
Thymus					
Absolute Weight (g)	0.6659	47*	0.1893	191*	
Body Weight Ratio (%)	0.1001	49*	0.0513	150*	
Brain Weight Ratio (%)	28.3915	46*	9.0415	190*	

* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for MYK-461-treated groups expressed as percentage control mean value.

Histopathology: Test substance-related findings were noted exclusively in the heart in females at ≥0.6 and in males at 1.2 mg/kg/day. The adverse finding of osseous/cartilaginous metaplasia of the left ventricular muscular bands, apex of the heart was of uncertain etiology (Table 74). It was expanded by osteocytes embedded in osteoid or chondrocytes within lacunae embedded in a basophilic acellular ground substance (cartilage matrix). The region variably contained mineralized debris and were accompanied by a low number of mononuclear cells, cell debris, and fibroblasts. An unscheduled high dose female (#B52515)

had these heart findings in addition to an atrial thrombus. Based on the histopathological findings in the heart, the NOAEL was 0.3 mg/kg/day.

Table 74. Test article-related microscopic findings in the heart in rats dosed with MYK-461 for 26 weeks (scheduled necropsy) -Terminal euthanasia

	MYK-461							
Sex	Males				Females			
Dose Level (mg/kg/day)	0	0.30	0.6	1.2	0	0.30	0.6	1.2
Number Examined	10	9	9	10	10	10	10	9
Heart								
Metaplasia, osseous/cartilaginous, left ventricular muscular bands, apex								
Minimal	0	0	0	2	0	0	1	2
Slight	0	0	0	1	0	0	1	2

Similar heart findings were observed in one recovery male. The sponsor notes that assessing reversibility of this lesion was difficult because once formed, bone and cartilage do not spontaneously revert to original muscle or fibrous connective tissue, and thus it should be considered permanent.

Toxicokinetics: Following oral administration of MYK-461, peak concentration reached between 0.5 and 2 hr post dose. Systemic exposures (Cmax and AUC) increased approximately proportionately to the increase in dose on all days of measurement. There was no sex-dependent difference in systemic exposure. Accumulation (in the range of 3.5 to 8.3) was noted after repeated daily oral doses. The data suggest that the steady state was reached prior to day 91 and was maintained through day 182 of treatment (Table 75).

Table 75. Summary of MYK-461 exposure data in a 26-week oral study in rats

Interval	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	AUC_{0-24} (ng·hr/mL)
Day 1	0.30	M	29.4	411
		F	32.4	483
		MF	29.8	447
	0.6	M	58.2	881
		F	54.3	851
		MF	52.5	866
	1.2	M	138	1660
		F	112	1820
		MF	119	1740
Day 91 (Week 13)	0.30	M	123	1370
		F	135	1780
		MF	129	1570
	0.6	M	448	5970
		F	323	4260
		MF	385	5110
	1.2	M	477	6520
		F	764	11800
		MF	621	9150
Day 182 (Week 26)	0.30	M	105	1470
		F	141	1870
		MF	120	1670
	0.6	M	547	6910
		F	369	4860
		MF	436	5890
	1.2	M	640	8490
		F	812	12900
		MF	725	10700

AUC_{0-24} = Area under the concentration time curve from hour 0 to hour 24; C_{max} = Maximum observed concentration; F = Female; M = Male; MF = Male and Female combined.

6.2.6 Six-week oral toxicity study in dogs with a 4-week recovery period

Conducting laboratory and location: (b) (4)

Sponsor Study number: NC-15-0009
Testing Facility Study Number: 14-3222
Date of study initiation: March 24, 2014
Date of last necropsy: June 28, 2014
Drug, Lot number: MYK-461, 400-13-01-53, 99.6% purity
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

At the 3 mg MYK-461/kg/day high-dose level, 7/12 dogs died or were euthanized after receiving 6 or 7 doses. The dosing of the group was terminated. The second high dose, 1 mg/kg/day, was also not tolerated; 8/12 dogs were euthanized after 25/26 doses and dosing was terminated. Ante-mortem clinical signs noted at these dose levels included pale gums, prolonged capillary refill time, decreased activity, labored/rapid breathing and pallor. Some animals were noted for body weight loss and decreased food consumption prior to death. The major factor contributing to the moribundity/mortality was cardiac toxicity resulting in heart failure. Histopathologic findings were noted in the heart, lungs, lymph nodes and thymus in some animals at both dose levels. Edema and congestion were present in multiple organs and body cavities of unscheduled decedents. Decreased cellularity in the thymus was noted at all dose levels with partial recovery in animals receiving 1 mg/kg/day. Based on these findings, the NOAEL was 0.3 mg/kg/day.

Purpose

The primary objective of the study was to evaluate the toxicity, toxicokinetic profile and the no observed adverse effect level of MYK-461 when administered orally to dogs, once daily, for 6 weeks, and to estimate the potential for reversal of any toxic effects within a 4-week drug-free period.

Methods

Formulation

Test substance was dissolved in 0.5% methylcellulose in distilled water. Drug formulations were prepared weekly and stored at room temperature. The prepared dosing formulations were evaluated for homogeneity (day 1 and day 25) and concentration on the day of dose preparations for day 4, and weeks 1, 2 and 7. Analyses of the formulations demonstrated that MYK-461 in 0.5%

Methylcellulose in distilled water was stable when stored at ambient temperature for 10 days.

Animals

Species/Strain: Beagle dogs from (b) (4)

#/Sex/Group: 4; an additional 2 dogs/sex in the control and top two high dose groups were designated for the recovery phase (Table 76).

Age: 12 months at start of dosing

Weight: Males: 9.7 to 11.6 kg; Females: 5.7 to 8.1 kg at the start of dosing

Husbandry: Animals were housed 2 or 3 of the same sex per cage. Dogs were fed with a measurable quantity of certified dog diet daily except for at least 12 hr prior to blood collection for clinical pathology and necropsy. Water was available *ad libitum* (except during the period of urine collection).

Dosing

Doses: Male and female dogs (26 each) were randomly assigned to five dose groups (Table 76). Dose levels were based on the results of a previous 4-week exploratory oral toxicity study (# (b) (4) 13-1261) in which dogs received an oral dose of 0.4, 1.3 or 4 mg/kg/day (n = 2/sex/dose) for 28 consecutive days. On day 13, a female dog was found dead in her cage with no abnormal clinical observations. On day 14, abnormal clinical signs including emesis and hypoactivity were noted in both male dogs. Subsequently a male dog died on the same day and the surviving male and female dogs was euthanized on day 15, thus terminating the high dose group. Gross and microscopic findings in the lungs characterized primarily by pulmonary edema were observed in unscheduled euthanized dogs. There was no effect of test substance at 0.4 and 1.3 mg/kg/day.

Table 76. Study design

Group	MYK-461 Dose (mg/kg)	Dose Formulation Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals			
				Main Study		Recovery Study	
1	0	0	10	4	4	2	2
2	0.1	0.01		4	4	0	0
3	0.3	0.03		4	4	0	0
4a	1	0.1		4	4	2	2
5b	3	0.3		4	4	2	2

M = male(s); F = female(s)

Dosing of Group 4 animals was discontinued on Day 28/29 and all surviving main study Group 4 animals were euthanized. Group 4 recovery animals were continued on study with no further dosing.

[a] All except 2 male and 3 female Group 5 animals were either found dead or euthanized for welfare reasons on Day 7/6. Dosing of 5 remaining Group 5 animals (who remained on study through termination of dosing) was discontinued on Day8/7.

Mode and Duration of Administration: Once daily orally by gavage for all groups for 6 weeks. Control animals received drug vehicle (10 ml/kg). Recovery group animals were observed for an additional 4 weeks with no drug treatment.

Observations and Measurements

Clinical Signs: All main study animals were observed twice daily for mortality and clinical signs. Observations were also made prior to dose and between 0.5 and 1h post dose for poor health or toxic effects.

Body Weights: Recorded three times pretest, weekly during treatment and recovery phase for all dogs. Terminal body weights were recorded prior to scheduled necropsies.

Food Consumption: Recorded daily starting pretest week -1.

Ophthalmoscopy: Conducted on all animals pretest and at study termination.

ECG: Individual ECGs were taken for all surviving unanesthetized dogs at the end of dosing and at the end of recovery.

Hematology[1] and Clinical Chemistry[2]: Blood samples were collected from jugular or cephalic veins from all unanesthetized dogs in groups 1 to 3 and in recovery groups 4 and 5 pretest and at scheduled necropsy. Blood samples were also collected from animals sacrificed in moribund condition or for welfare reasons and from surviving animals in groups 4 and 5 at termination. Animals were fasted and water-deprived during the collection period.

Urinalysis[3]: Urine samples were collected for all dogs overnight pretest and scheduled necropsy.

Gross Pathology: A complete necropsy including macroscopic examination of abnormalities and collection of tissues from an extensive list (Table 77) was done on all animals including unscheduled decedents. All surviving animals were fasted overnight prior to scheduled necropsy.

Organs Weighed: The terminal body weights and weights of the organ collected (adrenal, brain, heart, epididymides, prostate, kidneys, liver, pituitary, ovaries, uterus, spleen, testes, thymus and thyroid) were recorded for all surviving animals at study termination at the scheduled necropsy.

Histopathology: Microscopic examination was performed on all tissues listed in Table 77 from all animals in all dose groups.

[1] erythrocytes, hematocrit, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, thrombocytes, red cell distribution width, white blood cell (total and differential), activated partial thromboplastin time, prothrombin time

[2] ALT, AST, AP, total bilirubin, total protein, albumin, glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, cholesterol, triglycerides, γ-glutamyltransferase

[3] microscopic examination, pH, specific gravity, volume, bilirubin, occult blood, glucose, ketone, protein.

Table 77. Tissues/organs sampled for histopathological examination

ORGAN NAME	WEIGHED	PRESERVED	EXAMINED MICROSCOPICALLY
animal identity		X	
adrenal glands	X	X	X
aorta (thoracic)		X	X
bone marrow smear (rib)		X	
bone (femur)		X	X
bone (sternum)		X	X
bone marrow (sternum, femur)		X	X[a]
brain	X	X	X
cecum		X	X
colon		X	X
diaphragm		X	X
duodenum		X	X
epididymides	X	X	X
esophagus		X	X
eyes		X	X
gallbladder		X	X
heart	X	X	X
ileum		X	X
jejunum		X	X
kidneys	X	X	X
lacrimal glands		X	X
liver	X	X	X
lungs and bronchi		X	X
lymph nodes (axillary)		X	X
lymph nodes (mesenteric)		X	X
mammary gland		X	X[b]
nerve (optic)		X	
nerve (sciatic)		X	X
ovaries	X	X	X
pancreas		X	X
Peyer's patches/GALT		X	X[c]
pituitary gland	X	X	X
prostate gland	X	X	X
rectum		X	
salivary glands (mandibular)		X	X
skeletal muscle (longissimus dorsi-posture)		X	X
skeletal muscle (quadracepts)		X	X
skeletal muscle (*rectus femoris*)		X	X
skin and subcutis (dorsal – base of tail)		X	X
spinal cord (cervical, thoracic, lumbar)		X	X
spleen	X	X	X
stomach		X	X
testes	X	X	X
thymus	X	X	X
thyroid/parathyroid glands	X	X	X
tongue		X	X
trachea		X	X
urinary bladder		X	X
uterus and cervix	X	X	X
vagina		X	X
tissues with macroscopic findings including tissue masses		X	X

[a]Qualitative examination of the bone marrow sections were performed.
[b]Females only
[c]GALT was evaluated only when present in routine sections.

Toxicokinetics: Blood samples for test substance determination were collected from all surviving dogs from jugular or cephalic veins prior to dosing (on day 38 only) and at 0.5, 1, 2, 4, 8 and 24 hr post dose on study days 1 (all groups) and 38 (except groups 4 and 5). Unscheduled blood samples were collected from animals receiving 3 mg/kg/day on day 9/10, and from animals receiving 1 mg/kg/day on days 27/28/29. The animals were not fasted prior to blood collection.

Results

Analysis of Formulations: The preparations produced homogeneous mixtures. The preparations were stable for up to 10 days at room temperature. The achieved concentrations of MYK-461 for mid and upper two doses, prepared on days 1, 4 and weeks 2 and 6 were within target and ranged from 94.7% to 106.9% of nominal. On the other hand, the achieved concentrations for the low dose varied between 76.7 and 97.9% of target suggesting some concentrations were outside the acceptance criteria. The sponsor states that these were considered inherent to the very low concentrations of the formulations resulting in variability a) when stock solution is diluted to lower concentrations and b) variability between each sample collected for analysis. Further, they advance the argument that this is because a difference of few particles in each sample will result in differences in the analytical results. Such variability in the analytical results of the samples, according to the study, did not affect the integrity of the study because dose-related test article effects were noted, and plasma concentrations indicated expected exposures.

Mortality: The high dose, 3 mg/kg/day, was not tolerated. After 6 or 7 doses, 6 (3 females, 3 males) of the 12 animals were euthanized and a male (#5032) was found dead. The dosing of this group was terminated on day 7. The remaining 5 survivors (2 males, 3 females) were held until the scheduled week 6 terminal necropsy. The major factor contributing to moribundity/mortality in this group was cardiac toxicity resulting in heart failure. The 2nd high dose, 1 mg/kg/day, was also not tolerated. After 25 or 26 doses, 8 (4 males, 4 females) of 12 animals were euthanized and dosing was terminated. The remaining 4 animals were held until the scheduled recovery necropsy at week 10. The major factor contributing to moribundity/mortality of 3 males in this group was cardiac toxicity resulting in heart failure (Table 78).

Clinical Signs: No test substance-related clinical signs were noted in the low and mid dose groups. Ante-mortem clinical signs noted in one or more animals at the 1 and/or 3 mg/kg/day dose levels included: pale gums; prolonged capillary refill time; decreased activity; labored/rapid breathing; and pallor.

Table 78. Mortality[a]

Dose (mg/kg/day)	Animal # and sex	Day of death/ euthanasia	Mortality details
3	5030M, 5031M, 5032M, 5033M, 5530F, 5533F, and 5534F	6/7	All were euthanized except #5032M that was found dead. Clinical signs noted between the onset and euthanasia were: pale gums, pale appearance, cold to touch, and/or decreased activity, ptosis.
1	4030M	26	Euthanized with clinical signs of decreased activity, labored breathing, pale gums, prolonged CRT ~5 seconds, unresponsiveness and cold to touch
1	4034M, 4035M	28	Euthanized due to decreased activity, increased respiration rate and /or increased rate and force of exhaled breaths.
1	4033M, 4530F, 4531F, 4532F, and 4533F	28/29	Group terminated by euthanizing all surviving animals. No test substance-related clinical signs were noted in these animals.

a: The major factor contributing to death of all these animals (1.0 and 3.0 mg/kg/day dose groups) was cardiac failure.

Body Weights: In the high dose group, two males (5032, 5033) that died prematurely on day 6/7, showed a body weight loss of 0.2 and 0.4 kg, respectively, relative to pretest values. Similarly, loss of body weight was noted in male unscheduled decedents (4034 and 4035 showed a loss of 0.9 and 0.6 kg, respectively) receiving 1 mg/kg/day. Surviving animals in the top two high dose groups showed no effect on body weights. In addition, there were no test substance-related effects on mean body weight or body weight gain in the low and mid dose groups.

Food Consumption: Either a decrease (<50%) or no change in food consumption (for 1 or 2 days) was noted prior to onset of clinical signs in the unscheduled decedents. Normal food consumption was noted in mid and low dose groups.

Ophthalmoscopy: No remarkable treatment-related ocular changes.

ECG: Because of early termination of two high dose groups, only animals in the low and mid dose groups were evaluated. There was no effect of test substance on ECG in these animals.

Hematology: Test substance-related effects were noted in unscheduled deaths/early terminations in groups receiving ≥1 mg/kg/day. Administration of MYK-461 at 3 mg/kg/day for 6/7 days was associated with moderate increases in red cell mass (hemoglobin, hematocrit, and red blood cells), reticulocyte, white blood cells, neutrophils and monocytes (Table 79). The increases in neutrophils and monocytes, according to the sponsor, are considered likely to be secondary to inflammation. In animals receiving 25/26 doses of 1 mg/kg/day, there were

slight increases in reticulocytes and red cell distribution and decreases in red cell mass (Table 80). According to the sponsor, this is considered to reflect regenerative responses secondary to decreased tissue perfusion/oxygenation. There were no test substance-related effects on hematology in animals receiving 0.1 or 0.3 mg/kg/day

Table 79. MYK-461-related hematology changes (relative to pretest values) in animals administered 3 mg/kg/day for 6/7 Days.

Sex		Males		Females
Dose (mg/kg/day)	3	3	3	3
Doses Administered	7	7	7	6
Day of Collection	7	7	7	6
Animal no.	5030	5031	5033	5530
Hemoglobin (HGB)	-	+20%	+41%	-
Hematocrit (HCT)	-20%	+16%	+39%	-
Red Blood Cells (RBC)	-20%	+17%	+34%	-
Reticulocytes	-	3.2X	7.9X	2.4X
Mean Cell Hemoglobin (MCH)	+25%	-	-	-
Mean Cell Hemoglobin Concentration (MCHC)	+25%	-	-	-
White Blood Cells (WBC)	-	-	2.5X	+74%
Neutrophils	-	-	3.4X	2.2X
Monocytes	-	-	4.1X	+67%

Table 80. MYK-461-related hematology changes (relative to pretest values) in animals receiving 1 mg/kg/day for 25/26 days followed by a 2-day dose-free period.

Sex		Males		Females	
Dose (mg/kg/day)	1	1	1	1	1
Doses Administered	26	26	26	25	25
Day of Collection	29	28	28	28	28
Animal	4033	4034	4035	4530	4533
HGB	-14%	-	-	-	-18%
HCT	-16%	-	-	-	-18%
RBC	-13%	-	-	-	-17%
Reticulocytes	2.5X	-	2.5X	3.7X	-
RDW	-	-	+7.6%	-	-

Clinical Chemistry: Test substance-related moderate increases in ALT and AST relative to control animals that correlated with liver congestion were noted in unscheduled decedents in animals receiving 3 (Table 81) and 1 (Table 82) mg/kg/day. BUN, creatinine, potassium and phosphorus were also elevated in some of the decedents reflecting pre-renal azotemia. There were no test

substance-related clinical chemistry findings in recovery period and in low and mid dose group animals.

Table 81. MYK-461-related clinical chemistry changes (versus pretest) in animals administered 3 mg/kg/day for 6/7 days.

Sex	Males			Females		
Dose (mg/kg/day)	3	3	3	3	3	3
Doses Administered	7	7	7	6	6	6
Day of Collection	7	7	7	6	6	6
Animal	5030	5031	5033	5530	5533	5534
Aspartate Aminotransferase (AST)	-	+72%	2.6X	-	-	-
Alanine Aminotransferase (ALT)	-	+92%	3.8X	-	-	-
Urea Nitrogen (BUN)	2.1X	+38%	+76%	+83%	-	+32%
Creatinine	+33%	-	+29%	-	-	-
Potassium (K)	-	-	-	+20%	+16%	-
Chloride	-	-	-6.1%	-	-	-
Total Calcium	-	-	-5.9%	-	-	-
Phosphorus	+52%	-	+46%	-	+36%	+33%

Table 82. MYK-461-related clinical chemistry changes (versus pretest) in animals administered 1 mg/kg/day for 25/26 days followed by a 2-day dose-free period.

Sex	Males	
Dose (mg/kg/day)	1	1
Doses Administered	26	26
Day of Collection	28	28
Animal	4034	4035
Aspartate Aminotransferase (AST)	2.2X	2.2X
Alanine Aminotransferase (ALT)	4.8X	4.1X
Urea Nitrogen (BUN)	+47%	+50%
Total Calcium	-9.8%	-4.2%

Urine Analysis: No treatment related effects.
Gross Pathology: No test article-related macroscopic findings noted in animals necropsied at term. Although the death for all unscheduled decedents was MYK-461-related cardiac toxicity resulting in heart failure, no macroscopic changes in the heart was noted, because heart failure is a functional condition that is associated with minimal or no morphologic changes in the heart tissue.
Organ Weights: Low thymus weights relative to control were noted in females receiving ≥0.1 mg/kg/day and in males receiving 0.3 mg/kg/day (Table 81). Low

body weights correlated macroscopically with small thymuses and microscopically with decreased cellularity (see below and Table 84).

Table 83. MYK-461-related organ weight changes in the thymus (% difference relative to controls) in dogs dosed with MYK-461 for 6 weeks

Group/sex Dose (mg/kg/day)	2M 0.1	3M 0.3	2F 0.1	3F 0.3
Thymus				
Absolute weight (%)	-	-32	-24	-42[a]
vs. body weight (%)	-	-32	-28	-44[a]
vs. brain weight (%)	-	-35	-24	-45[a]
Number of animals examined	4	4	4	4

[a]Statistically significant difference between mean values for test article-treated and control groups.
- = not test article-related.

Histopathology: Test substance-related findings were noted in the heart, thymus, mesenteric lymph node, spleen and prostate in a few males and/or females unscheduled decedents receiving 1 or 3 mg/kg/day (Table 84). Microscopic findings in the heart consisted of minimal hemorrhage, inflammatory cell infiltrates, and slight myxomatous change and edema in the atrioventricular valve, minimal inflammatory cell infiltrates in the epicardium and minimal degenerative changes in some coronary vessels. Lungs showed minimal to slight alveolar inflammation and/or increased alveolar macrophages and minimal lymphocyte apoptosis in the perivascular inflammatory cell infiltrates. The thymus and the lymph node showed a moderate decrease in lymphocyte cellularity. Abnormalities noted only at the 3 mg/kg/day high-dose level were slight to marked apoptosis in the spleen and moderate to marked acinar atrophy in the prostate. Findings considered secondary to MYK-461-related heart failure were edema and congestion that were present in multiple organs and body cavities such as gallbladder (correlated macroscopically with thickened, pale gallbladder walls), thymus (in and around), pancreas, pericardium and mesenteric lymph node, brain and lungs. Thin red fluid was present in the abdominal cavity and the trachea. Congestion was noted in the liver, stomach and rectum (correlated macroscopically with dark areas). MYK-461-related findings were also present in the heart in early termination animals receiving 1 mg/kg/day. Test substance-related microscopic findings in animals receiving 0.1 or 0.3 mg/kg/day were limited to decreased cellularity in the thymus (Table 84). Because of the magnitude of the change relative to controls, and correlation with lower organ weights and small size, the findings are attributed to test substance administration. A partial recovery in lymphocyte cellularity in the thymus was

noted in 2 males and 2 females receiving 1 mg/kg/day. This finding correlated with low thymic weights in recovery females compared to controls (absolute and relative to body and brain weights of -24%, -14% and -28% respectively).

Table 84. MYK-461-related findings in the thymus, heart and other organs in dogs dosed with MYK-461 for 6 weeks

	0.00		0.1		0.3		1.0		3.0	
Number of Animals	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Thymus, decreased cellularity, minimal	0	2	2	1	0	9	0	0	1	0
Thymus, decreased cellularity, slight	3	2	1	3	3	3	0	0	2	1
Thymus, decreased cellularity, moderate	0	0	0	0	1	1	2	0	0	1
Thymus, decreased cellularity, marked	0	0	1	0	0	0	1	0	0	0
Heart-hemorrhage, valve	0	0	0	0	0	0	0	0	2	1
Heart, infiltrate, valve	0	0	0	0	0	0	1	2	0	2
Heart-edema, valve, pericardium	0	0	0	0	0	0	2	0	1	0
Heart-epicardium, infiltrate	0	0	0	0	0	0	1	0	0	2
Heart-coronary vessel, degeneration	0	0	0	0	0	0	0	0	1	0
Lungs, increased alveolar macrophages	0	0	0	0	0	0	3	0	0	0
Lymph nodes, cellularity decreased	0	0	0	0	0	0	1	0	1	0
Spleen, apoptosis, white pulp	0	0	0	0	0	0	0	0	3	3
Prostate, colloid reduced	0	0	0	0	0	0	0	0	2	0

Toxicokinetics: Both Cmax and AUC values increased with increasing doses over the dose range of 0.1 to 3 mg/kg/day on treatment day 1 and over the dose range 0.1 to 0.3 mg/kg/day during 6 weeks of treatment. The increase was less than the proportionate dose increment. The systemic exposure of female dogs to test substance was generally similar to that of male dogs. Accumulation (an average increase of 3- to 9-fold in Cmax and AUC values) was noted with repeated dosing in both males and females. Interconversion of MYK-461 to its enantiomer, MYK-460, after either single-dose or repeat-dose administration was minimal (Table 85).

Table 85. Summary of toxicokinetic parameters of MYK-461 in dogs treated orally with MYK-461

Dose (mg/kg/day)	C_{max} (ng/mL)				AUC_{0-24} (ng·h/mL)			
	Day 1		Week 6		Day 1		Week 6	
	M	F	M	F	M	F	M	F
MYK-461								
0.1	33.4	33.6	88.9	110	220	239	1690	1480
0.3	60.1	56.1	303	220	621	553	5900	3700
1	150	178	107[a]	53.5[a]	1720	1630	2330[a]	1050[a]
3	296	370	6.71[b]	-[b]	3400	4850	151[b]	-[b]
MYK-460								
0.1	0.451	0.426	0.504	0.450	1.71	1.23	2.11	1.52
0.3	0.959	0.840	1.49	1.18	8.89	5.24	13.0	8.95
1	2.59	2.14	-a	-a	23.9	18.2	-a	-a
3	4.54	5.80	-b	-b	45.9	59.0	-b	-b

M = male(s); F = female(s);

[a] Animals received treatment for 25/26 days, then treatment was suspended

[b] Animal received treatment for 6 or 7 days, then treatment was suspended

6.2.7. 3-Month oral toxicity study in dogs with an 8- or 11-week recovery

Conducting laboratory and location: [redacted] (b) (4)

Sponsor Study number: NC-19-0051
Testing Facility Study Number: TXC1550
Date of study initiation: April 21, 2015
Date of last necropsy: August 18, 2015
Drug, Lot number: MYK-461, 15B0013 ([redacted] (b) (4) lot 150030), 99.87% purity
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

At the 0.45 mg MYK-461/kg/day high-dose level, 2/12 dogs died or were euthanized on day 70/72. The dosing of the males for this group was terminated. The euthanized animal demonstrated decreased activity and labored respiration. Cardiac toxicity (severe ventricular dilation) resulting in heart failure was the major factor contributing to the moribundity/mortality. A high dose female terminally euthanized also showed ventricular dilation of the heart. At the end of the recovery period, no test substance-related changes were observed. ECG evaluation showed a marked increase in heart rate associated with an increase in R and T amplitudes in a high dose male at the end

of the dosing period. A slight prolongation of QT and QTc intervals was observed in few animals at ≥ 0.18 mg/kg/day and was considered non-adverse event by the sponsor. According to the sponsor, the NOAEL was 0.3 mg/kg/day.

Purpose

The primary objective of the study was to evaluate the toxicity, toxicokinetic profile and the no observed adverse effect level of MYK-461 when administered orally to dogs, once daily, for 3 months, and to estimate the potential for reversal of any toxic effects within a 4-week drug-free period.

Methods

Formulation

Test substance was dissolved in 0.5% methylcellulose in distilled water. The frequency of preparation of drug formulations is not given. The prepared dosing formulations were evaluated for concentration on the day of dose preparations for day 1, and weeks 6 and 13. Analyses of the formulations demonstrated that MYK-461 in 0.5% Methylcellulose in distilled water was stable when stored at ambient temperature or refrigerated condition for 2 weeks.

Animals

Species/Strain: Beagle dogs from (b) (4)

#/Sex/Group: 4; an additional 2 dogs/sex in the control and top two high dose groups were designated for the recovery phase (Table 86).

Age: 10-12 months at start of dosing

Weight: Males: 7.6 to 11.05 kg; Females: 6.5 to 9.55 kg at the start of dosing

Husbandry: Animals were housed individually in cages. Dogs were fed with a measurable quantity of certified dog diet daily except for at least 16-20 h prior to blood collection for clinical pathology and necropsy. Water was available *ad libitum*.

Dosing

Doses: Male and female dogs (26 each) were randomly assigned to five dose groups (Table 86). Dose levels were based on the results of a previous 6-week oral toxicity study in dogs at doses of 0.1, 0.3, 1 and 3 mg/kg/day (section 6.2.6). In this study, early mortality was noted at 1 and 3 mg/kg/day resulting in termination of dosing. Cardiac toxicity resulting in heart failure was the major factor contributing to the moribundity/mortality. The NOAEL in this study was 0.3 mg/kg/day.

Mode and Duration of Administration: Once daily orally by gavage for first 4 groups for 13 weeks and the fifth group males (0.45 mg/kg/day) for 73 days. Control animals received drug vehicle (5 ml/kg). Two additional animals per sex each in the control and at 0.45 mg/kg/day were maintained free of drug for an

additional 11 weeks (since dosing was stopped for this group on Day 73). The 2nd high dose group, 0.3 mg/kg/day, was maintained free of drug for 8 weeks.

Table 86. Study design

Group	Dosage (mg/kg/day)	Number/sex	Animal identity number	
			Male	Female
1[a]	0	4 + 2 in recovery[b]	1-6	7-12
2	0.06	4	13-16	17-20
3	0.18	4	21-24	25-28
4	0.30	4 + 2 in recovery[b]	29-34	35-40
5	0.45	4 + 2 in recovery[b]	41-46	47-52

a Control group (vehicle: methylcellulose/water (0.5/99.5%))

b The first two males and the first two females from Groups 1, 4 and 5 were kept for recovery

The last day of dosing was on Day 73 for males from Group 5 (including recovery animals) and terminal necropsy was on day 74 following the death and euthanasia for humane reason of 2 males from this dose group. For all other groups (excluding 2 high dose recovery animals) including the high dose females, the last day of dosing was Day 92 or 93. Recovery phase animals were terminated on Day 150 for males (11 weeks recovery for the 2 high dose males as the dosing was stopped on Day 73) and on Day 149 for females from groups 1, 4 and 5 (8 weeks recovery).

Observations and Measurements

Clinical Signs: All animals were observed 3 times a day for mortality and clinical signs during the dosing phase and once daily during the recovery phase.

Body Weights: Recorded weekly during treatment and recovery phase for all dogs. Terminal body weights were recorded prior to scheduled necropsies.

Food Consumption: Recorded daily starting pretest week -1.

Ophthalmoscopy: Conducted on all animals pretest and on study day 88/89.

ECG: Individual ECGs were taken for all surviving unanesthetized dogs pretest and on day 73 (group #5), day 77 (males from Groups 1, 2, 3 and 4), Day 75 (females from Groups 1, 2, 3, 4 and 5) and at the end of recovery (day 147 for females from groups 1, 4 and 5; day 149 for males from groups 1, 4 and 5).

Hematology[4] and Clinical Chemistry[5]: Blood samples were collected from jugular or cephalic veins from all unanesthetized fasted dogs pretest, day 36, day 74 (remaining males from group 5 since dosing for this group was terminated after 73rd dose), day 86 and in recovery groups on day 148 (females from groups 1, 4 and 5) and day 150 (males from groups 1, 4 and 5). Blood sample was

[4] erythrocytes, hematocrit, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, thrombocytes, red cell distribution width, white blood cell (total and differential), fibrinogen, activated partial thromboplastin time, prothrombin time

[5] ALT, AST, AP, γ-glutamyltransferase, creatine kinase, total bilirubin, total protein, albumin, globulin, A/G ratio, glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, cholesterol, triglycerides,

collected from the euthanized high dose male (#45) on day 72. For biomarkers (Troponin I (cTnI) and N-terminal pro B-type natriuretic peptide (NT-proBNP)) analyses, blood samples were collected pretest, and 4 hr after dosing on study day 7 (all animals), 36 (all animals), 70 (females), 72 (males), 86 (all animals except males from group 5), day 148 (females from groups 1, 4 and 5) and day 150 (males from groups 1, 4 and 5)

Urinalysis[6]: Urine samples were collected for all dogs fasted overnight (allowed water) pretest and on day 36 (males and females, except for few animals for which the urine was collected on day 38), day 74 (males from group 5), day 91 (females from groups 1, 2, 3, 4 and 5) or day 92 (males from groups 1, 2, 3 and 4), and day 148 (females from groups 1, 4 and 5) or day 150 (males from groups 1, 4 and 5).

Gross Pathology: A complete necropsy including macroscopic examination of abnormalities and collection of tissues from an extensive list (Table 87) was done on all animals including unscheduled decedents. All surviving animals were fasted overnight and weighed prior to scheduled necropsy. Urine sample was not collected from the euthanized animal.

Organs Weighed: The terminal body weights and weights of the organ collected (adrenal, brain, heart, epididymides, prostate, kidneys, liver, pituitary, ovaries, uterus, spleen, testes, thymus and thyroid) were recorded for all surviving animals at study termination at the scheduled necropsy.

Histopathology: Microscopic examination was performed on all tissues listed in Table 87 from all animals in all dose groups.

Toxicokinetics: Blood samples for test substance determination were collected from all surviving non-fasted dogs from jugular vein at 0.25, 0.5, 1, 2, 4, 8 and 24 h post dose on study days 1, 36 (all groups), day 73 (males from group 5), day 86 (all animals, except males from group 5) and at necropsy (all animals, except males from group 5). One sample per animal was collected during the recovery period, days 87, 102, 108, 122, 124, 130 and 150. At the time of necropsy (including recovery period) (except for a high dose male (#45M), one piece of left ventricle, medial head of the triceps brachii and long head of the triceps brachii were collected from each animal for test substance determination.

[6] microscopic examination, pH, specific gravity, volume, bilirubin, occult blood, glucose, ketone, protein.

Table 87. Tissues/organs sampled for histopathological examination

Tissue collection	Organ weights	Microscopic examination
Tissues with macroscopic observations (including masses)		X
Adrenal gland (2)	X	X
Aorta		X
Bone: femur [a]		X
Bone: sternum [b]		X
Bone marrow: femur [a]		X
Bone marrow: sternum [b]		X
Brain	X	X
Epididymis (2) [c]	X	X
Esophagus		X
Eye (2) [c]		X
Gallbladder		X
Heart [d]	X	X
Intestine: duodenum		X
Intestine: jejunum		X
Intestine: ileum		X
Intestine: cecum		X
Intestine: colon		X
Intestine: rectum		X
Intestine: gut associated lymphoid tissue (Peyer's patch) [e]		X
Joint: femorotibial		X
Kidney (2)	X	X
Lacrimal gland (2)		X
Larynx [f]		
Liver	X [g]	X
Lung (with bronchus)	X	X
Lymph node: mesenteric		X
Lymph node: retropharyngeal (2)		X
Mammary gland		X
Nasal tissue [f] (skull/nasal cavity)		
Nerve: optic (2) [c, h]		X
Nerve: sciatic		X
Ovary (2)	X [i]	X
Oviduct (2)		X
Pancreas		X

Tissues/organs sampled for histopathological examination (continued)

Tissue collection	Organ weights	Microscopic examination
Parathyroid gland (2) [h]		X
Pituitary gland	X	X
Prostate gland	X	X
Salivary gland: mandibular (2) [j]		X
Salivary gland: sublingual (2) [j]		X
Salivary gland: parotid (2) [j]		X
Skeletal muscle: diaphragm		X
Skeletal muscle: quadriceps femoris (cranial thigh)		X
Skeletal muscle : longissimus dorsi (2)		X
Skeletal muscle : gastrocnemius muscles (2)		X
Skin		X
Spinal cord (cervical, thoracic, lumbar)		X
Spleen		X
Stomach		X
Testis (2) [c]	X	X
Thymus	X	X
Thyroid gland (2 lobes)	X [k]	X
Trachea		X
Tongue		X
Ureter (2)		X
Uterus (body and horns)	X [l]	X
Uterus: cervix		X
Urinary bladder		X
Vagina		X

a Sampled with femorotibial joint
b Sampled as a whole
c Fixed in Davidson's fixative (except for animals found dead where they are preserved in 10% neutral buffered formalin)
d All 4 chamber free walls, papillary muscle, and interventricular septum, and atrioventricular and sinoatrial nodes, if possible.
e Peyer's patch included in section of ileum or jejunum
f Retained in fixative
g Liver weighed with gall bladder (opened and drained)
h Only one side required on slide
i Weighed without oviducts
j Collect two, process and examine one microscopically
k Weighed with parathyroid gland
l Body and horns weighed with cervix (without oviducts)
m (2) both sides are sampled for paired organs

Results

Analysis of Formulations: The achieved concentrations of MYK-461 for all formulations were within the ± 20% acceptance criteria of the nominal concentration.

Mortality: Two males receiving 0.45 mg/kg/day were either died (#44M, day 70) or euthanized (#45, day 72). The dead animal did not demonstrate any previous clinical signs. However, the euthanized animal on the same day demonstrated decreased activity and labored respiration. In addition, the euthanized animal showed an increase (7.9-fold relative to pre-test values) in NT pro-BNP. Both animals showed a moderate decrease (-4% for #44M, 7.4% for #45M) in body weight. The major factor contributing to moribundity/mortality was cardiac toxicity resulting in heart failure. Both high dose males (#43M, #46M) that were euthanized as scheduled on Day 74 (see Table 86) and 1 of the 2 females that were terminally euthanized on Day 93 showed macroscopic minimal or slight dilation of the heart, together with microscopic mononuclear cell subendothelial infiltration of the cardiac mitral valve.

Clinical Signs: No test substance-related clinical signs were noted in the first 3 dose groups. The survived high dose group animals showed decreased activity, thin in appearance, labored respiration on days 72-73.

Body Weights: A slight loss (-7.4% between days 1 and 71) in body weight was noted for high dose group males.

Food Consumption: No treatment-related changes were noted for any dose group.

Ophthalmoscopy: No remarkable treatment-related ocular changes.

ECG: A marked increase in heart rate relative to pretest values was noted in early decedents and survived high dose group animals and a female receiving 0.18 mg/kg/day. Slight QT, QTcv and QTcf prolongations were recorded in 2/6 females (51F and 52F) at 0.45 mg/kg/day (up to +8%, +7% and +9% in QT, QTcv and QTcf respectively, when compared with respective pre-treatment values), in 2/6 males (29M and 32M) at 0.3 mg/kg/day (up to +11%, +17% and +19% in QT, QTcv and QTcf respectively, when compared with respective pre-treatment values) and in 1/4 males (21M) at 0.18 mg/kg/day (+13%, +14% and +16% in QT, QTcv and QTcf respectively, when compared with respective pre-treatment values) on days 75 or 77, without any dose-relationship (Table 88). The sponsor does not consider these changes as adverse since, these increases were slight; the values remained close to the pretest or control values. This interpretation is not correct.

Hematology: No test substance-related effects were noted in any dose groups.

Clinical Chemistry: No effect of test substance on cardiac troponin values was noted. A slight increase (4.1- to 7.9-fold relative to pretest value) in NT pro-BNP was observed in both early decedents (#46M, #45M) on day 70/72. The values in treated animals in all other dose groups were comparable to pretest and control values.

Table 88. Heart rate and QT intervals after 90-day dosing with MYK-461 in dogs

Study Day	Heart Rate (bpm)		QT (milliseconds)		QTcf (milliseconds)	
	Male	Female	Male	Female	Male	Female
Group 1, Control	100	114	214	204	255	254
Group 2, 0.06 mg/kg	103 (+3)	102 (-11)	205 (-4)	211 (+3)	248 (-3)	255 (-)
Group 3, 0.18 mg/kg	90 (-10)	108 (-5)	222 (+4)	204 (-)	257 (+1)	247 (-3)
Group 4, 0.30 mg/kg	102 (+2)	106 (-7)	214 (-)	217 (+6)	258 (+1)	264 (+4)
Group 5, 0.45 mg/kg	132 (+32)	116 (+2)	201 (-6)	223 (+9)	260 (+2)	272* (+7)

*p<0.05, () = % different from control

Urine Analysis: No treatment related effects.
Gross Pathology: In both unscheduled decedents, the heart showed a marked/severe dilation of the left and right ventricles. In both animals, the trachea was filled with a large amount of pale froth material and the lung had a moderate, coalescent to diffuse red discoloration with non-collapsed lobes; these changes were regarded as secondary to MYK-461-related left-sided heart failure. In terminally euthanized animals, a high dose female (#51F), showed a minimal to slight dilation of the left and right ventricles as well a rounded shape. No macroscopic changes were noted in any of the recovery animals.
Organ Weights: No treatment-related effects.
Histopathology: Test substance-related microscopic changes were noted in the heart, pericardium, lungs, liver, thymus and gall bladder of both premature decedent animals. Minimal, focal to multifocal, mononuclear subendothelial infiltrates were noted in the mitral and tricuspid valves. Lungs showed marked, diffuse chronic congestion characterized by engorged alveolar capillaries, extravasated red blood cells and numerous alveolar macrophages containing red blood cells. Diffuse congestion was noted in the liver. Gall bladder, thymus and pericardium showed diffuse edema. MYK-461-related findings were also present in the heart of one female dosed 0.45 mg/kg/day and terminally euthanized. The heart showed slight dilation of both ventricles together with microscopic, mononuclear subendothelial infiltration of the cardiac mitral valve. No MYK-461-related pathological findings were noted in animals receiving 0.06, 0.18 and 0.30 mg/kg/day. Also, no microscopic findings that were considered related to the test substance administration were observed at the end of the recovery period. The NOAEL was 0.3 mg/kg/day.
Toxicokinetics: The systemic exposure (Cmax and AUC) increased approximately proportionately to the increase in dose on all days of measurement. Peak concentration was between 0.25 and 4 h post dosing (median 0.25 h post dosing). There was no sex-dependent difference in the

systemic exposure to MYK-461. Accumulation (in the range of 1.6 to 9.9) was noted after repeated daily oral doses. The data suggest that the steady state was reached on day 36 (Table 89). The mean enantiomer ratios (MYK-460 to MYK-461 ratio) for Cmax and AUC0-24 values were very low (<0.00068) (Table 90) suggesting a limited inter-conversion of the MYK-461 to MYK 460.

Table 89. Summary of toxicokinetic parameters of MYK-461 in dogs treated orally with MYK-461

Sex	Dose level (mg/kg/day)	Animal number	Day	C_{max}[a] (ng/mL)	t_{max}[b] (h)	AUC_{0-24}[a] (ng.h/mL)
Male	0.06	13-16	1	57.4 ±6.48 (11)	0.25 [0.25-0.25]	227 ±46.2 (20)
			36	119 ±29.4 (25)	0.38 [0.25-0.50]	1770 ±405 (23)
			86	121 ±18.7 (16)	0.25 [0.25-0.25]	1760 ±369 (21)
	0.18	21-24	1	193 ±46.1 (24)	0.25 [0.25-0.25]	754 ±168 (22)
			36	395 ±105 (27)	0.25 [0.25-0.50]	6290 ±2210 (35)
			86	388 ±100 (26)	0.25 [0.25-0.25]	6220 ±2280 (37)
	0.30	29-34	1	228 ±74.5 (33)	0.25 [0.25-0.25]	1070 ±359 (34)
			36	491 ±142 (29)	0.25 [0.25-0.50]	7490 ±1830 (24)
			86	496 ±124 (25)	0.25 [0.25-0.50]	7620 ±2010 (26)
	0.45	41-46	1	342 ±108 (32)	0.25 [0.25-0.50]	1820 ±614 (34)
			36	853 ±273 (32)	0.50 [0.25-0.50]	16000 ±5160 (32)
			73[c]	1040 ±390 (38)	0.38 [0.25-0.58]	17200 ±7430 (43)
Female	0.06	17-20	1	74.0 ±47.1 (64)	0.25 [0.25-0.50]	306 ±185 (61)
			36	104 ±48.6 (47)	0.38 [0.25-1.0]	1440 ±627 (43)
			86	120 ±49.8 (41)	0.25 [0.25-0.25]	1400 ±526 (38)
	0.18	25-28	1	167 ±58.4 (35)	0.25 [0.25-0.50]	742 ±177 (24)
			36	281 ±73.6 (26)	0.75 [0.50-1.0]	4380 ±1410 (32)
			86	288 ± 74.4 (26)	0.25 [0.25-0.25]	3800 ±1110 (29)
	0.30	35-40	1	267 ±70.4 (26)	0.25 [0.25-0.50]	1520 ±486 (32)
			36	617 ±159 (26)	0.25 [0.25-0.50]	9890 ±3180 (32)
			86	641 ±221 (34)	0.25 [0.25-0.25]	9550 ±4230 (44)
	0.45	47-52	1	293 ±84.9 (29)	0.25 [0.25-0.25]	1730 ±688 (40)
			36	755 ±243 (32)	0.38 [0.25-4.0]	12100 ±4390 (36)
			86	782 ±256 (33)	0.25 [0.25-0.25]	12300 ±4900 (40)

Values are rounded to 3 significant figures except for t_{max} values which are expressed with 2 significant figures. CV was expressed as integer.

a mean ± SD (CV%)

b median [min-max]

c animal 41,42, 43 and 46 collected on Day 73

Table 90. Summary of toxicokinetic parameters of MYK-460 (Enantiomer of MYK-461) in dogs treated orally with MYK-461

Sex	Dose level[d] (mg/kg/day)	Animal number	Day	C_{max}[a] (ng/mL)	t_{max}[b] (h)	AUC_{0-24}[a] (ng.h/mL)
Male	0.30	29-34	1	<LLOQ	1.0[1.0]	0.0489 ±0.120 (245)
			36	<LLOQ	0.50 [0.50-0.50]	0.105 ±0.163 (155)
			86	<LLOQ	NA	0.00± NA
	0.45	41-46	1	<LLOQ	1.0 [1.0-1.0]	0.686 ±0.623 (91)
			36	0.286 ±0.231 (81)	1.0 [1.0-1.0]	1.16 ±1.05 (90)
			73[c]	0.390 ±0.122 (31)	1.0 [0.50-2.0]	1.25 ±0.972 (78)
Female	0.30	35-40	1	<LLOQ	0.75 [0.50-1.0]	0.140 ±0.272 (194)
			36	<LLOQ	1.0 [0.50-1.0]	0.486 ±0.688 (142)
			86	<LLOQ	0.60 [0.58-1.0]	0.537 ±0.993 (185)
	0.45	47-52	1	<LLOQ	0.75 [0.50-1.0]	0.466 ±0.664 (143)
			36	0.260 ±0.204 (78)	1.0 [0.50-1.0]	0.875 ±0.807 (92)
			86	0.266 ±0.208 (78)	0.79 [0.50-1.0]	0.635 ±0.773 (122)

Values are rounded to 3 significant figures except for t_{max} values which are expressed with 2 significant figures. CV was expressed as integer number. NA : not applicable. LLOQ : lower limit of quantification.

a mean ± SD (CV%)

b median [min-max]

c animal 41,42, 43 and 46 collected on Day 73

d TK parameters not reported at 0.06 and 0.18 mg/kg/day dose levels, the compound being not quantified

MYK-461 was largely distributed in tissues (left ventricle, and, medial and long Triceps), the day after the last administration (days 92/93 or 74 in the high dose group) with mean tissue to plasma ratios ranging from 11 to 34, 9.9 to 34 and 6.5 to 26 in left ventricle, medial and long triceps, respectively. At the end of the recovery period (on Days 149/150, 57, 58 or 77 days after the last administration), test substance was not quantified in tissues investigated except in a high dose female (#48F) in the left ventricle (51.1 ng/g) (Table 91).

Table 91. Mean MYK-461 tissue concentrations and mean tissue to plasma ratio in dogs treated orally with MYK-461

			MYK-461 concentrations (ng/g)			Tissue to plasma concentration ratios		
Dose level (mg/kg/d)	Day	Sex	Left Ventricle	Medial Triceps	Long Triceps	Left Ventricle	Medial Triceps	Long Triceps
0.06	92	male	1290	1660	754	26	34	16
0.06	92	female	984	533	728	34	18	26
0.06	93	male	1330	1710	767	18	23	11
0.06	93	female	721	1010	841	11	16	13
0.18	92	male	4320	6320	3130	22	25	17
0.18	92	female	1800	1540	1930	14	12	16
0.18	93	male	3510	4860	2250	15	21	8.8
0.18	93	female	3150	4040	1800	22	29	13
0.30	92	male	6060	6420	3280	26	27	14
0.30	92	female	6400	4850	4840	15	9.9	13
0.30	93	male	5550	6600	3140	20	23	11
0.30	93	female	5150	6180	3140	15	17	8.8
0.30	150	male	<LLOQ	<LLOQ	<LLOQ	NA	NA	NA
0.30	149	female	<LLOQ	<LLOQ	<LLOQ	NA	NA	NA
0.45	74	male	5890	10500	5420	11	16	8.8
0.45	92	female	9390	6440	6130	19	15	12
0.45	93	female	8300	8650	4530	12	13	6.5
0.45	150	male	<LLOQ	<LLOQ	<LLOQ	NA	NA	NA
0.45	149	female	25.6	<LLOQ	<LLOQ	NA	NA	NA

LLOQ : lower limit of quantification, NA : not applicable

6.2.8. 39-Week oral toxicity study in dogs with a 17-week recovery

Conducting laboratory and location: (b) (4)

Sponsor Study number: NC16-0048
Testing Facility Study Number: 8334142
Date of study initiation: November 24, 2015
Date of last necropsy: January 6, 2017
Drug, Lot number: MYK-461, 15B0013 ((b) (4) lot 150030), 99.98% purity
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

All animals receiving 0.06, 0.18 or 0.45 mg/kg/day survived until their scheduled euthanasia. ECG evaluation showed a marked increase in heart rate associated with prolongation (11 to 35 msec) of QTc intervals in animals receiving ≥ 0.18 mg/kg/day relative to predose and control values. Mean QRS duration was prolonged (4 msec) in high dose females. The effects were reversed on day 26 of the recovery phase. Echocardiogram evaluations indicated substantial increases in mean LV end-diastolic and mean LV end-systolic volumes and a substantial reduction in mean LV ejection fraction in high dose males and females. The effects were noted by day 76 and became progressively more severe by day 256. A complete recovery was noted during the recovery phase. No test substance-related macroscopic or microscopic findings were noted at all dose levels. The changes in heart function at the high dose, characterized by alterations in both electrocardiography and echocardiography parameters, were considered adverse by the sponsor. Therefore, the no observed adverse effect level is 0.18 mg/kg/day. This corresponded to Cmax and AUC0-24 values (male and female combined) of 287 ng/mL and 4,680 ng·hr/mL, respectively on Day 273 of the dosing phase. However, the NOAEL for not causing QTc interval prolongation is 0.06 mg/kg/day.

Purpose

The primary objective of the study was to evaluate the toxicity, toxicokinetic profile and the no observed adverse effect level of MYK-461 when administered orally to dogs, once daily, for 39 weeks, and to estimate the potential for reversal of any toxic effects within a 17-week drug-free period.

Methods

Formulation

Test substance was dissolved in 0.5% methylcellulose in reverse osmosis water. Drug formulations were prepared once a week and stored in a refrigerator. The

dosing formulations prepared for administering during weeks 1, 5, 7, 8, 13, 21, 29 and 39 were evaluated for concentration verification.

Animals

Species/Strain: Beagle dogs from (b) (4)

#/Sex/Group: 4; an additional 2 dogs/sex in the control and the high dose group were designated for the recovery phase (Table 92).

Age: 6-7 months at start of dosing

Weight: Males: 7.4 to 10.0 kg; Females: 5.3 to 7.6 kg at the start of dosing

Husbandry: Animals were housed by sex in cages. Animals were acclimated to the test facility for 17 days prior to initiation of dosing. Dogs were fed for 4 to 5 hours each day except for at least 16-20 h prior to blood collection for clinical pathology and necropsy. Water was available *ad libitum*.

Dosing

Doses: Male and female dogs (26 each) were randomly assigned to four dose groups (Table 92).

Mode and Duration of Administration: Once daily orally by gavage for 39 weeks (273 days). Control animals received drug vehicle (5 ml/kg). Two additional animals per sex each in the control and the high dose group were maintained free of drug for an additional 17 weeks.

Table 92. Study design

Group[a]	No. of Animals[b] Male	Female	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
1 (Control)	6	6	0	0
2 (Low)	4	4	0.06	0.012
3 (Mid)	4	4	0.18	0.036
4 (High)	6	NA	0.30	0.06
4 (High)	NA	6	0.45	0.09

NA = Not applicable

a Group 1 was administered vehicle control article only.

b Animals designated for recovery euthanasia (two animals/sex in Groups 1 and 4) underwent at least 17 weeks of recovery following the last dosing interval.

Observations and Measurements

Clinical Signs: All animals were observed 2 times a day for mortality and clinical signs during the dosing phase and once daily during the recovery phase.

Body Weights: Recorded weekly during treatment phase for all dogs. Terminal body weights were recorded prior to scheduled necropsies.

Food Consumption: Recorded weekly from week 1 through week 15 of the dosing phase and every 4 weeks thereafter until the end of dosing. Also, measured during weeks 4, 8, 12 and 16 of the recovery phase.
Ophthalmoscopy: Conducted on all animals pretest and on study day 271 of the dosing phase.
ECG: Individual ECGs were recorded once pretest, once during week 39 of the dosing phase, and once during week 4 of the recovery phase. The heart rate-corrected QT (QTcf) interval was calculated using the Fridericia method
Echocardiogram Examinations: Echocardiograms were recorded twice during the predose phase (Day 8), during weeks 11, 23 and 37 of the dosing phase, and during weeks 4 and 12 of the recovery phase. Trans-mitral Doppler flow velocities were measured during diastole to include peak E and A wave velocities and their ratio. Animals were sedated with butorphanol and midazolam (0.3 ng/kg and 0.1 mg/kg, respectively, intramuscular).
Hematology[1] and Clinical Chemistry[2]: Blood samples were collected from jugular or cephalic veins from all fasted dogs twice pretest, during weeks 13 and 90 of the dosing phase, during week 5 of the recovery phase, and on the day of recovery euthanasia. For biomarkers (Troponin I (cTnI) and N-terminal pro B-type natriuretic peptide (NT-proBNP)) analyses, blood samples were collected twice pretest, during weeks 13 and 39 of the dosing phase, during week 5 of the recovery phase, and on the day of recovery euthanasia.
Urinalysis[3]: Urine samples were collected for all dogs fasted overnight once pretest, during weeks 13 and 59 of the dosing phase, during the week 5 of recovery phase, and on the day of recovery euthanasia.
Gross Pathology: A complete necropsy including macroscopic examination of abnormalities and collection of tissues from an extensive list (Table 91) was done on all animals on day 274 of the dosing phase (4 animals/sex/group) and on day 120 of the recovery phase (rest of the surviving animals). All animals were fasted overnight and weighed prior to scheduled necropsy.
Organs Weighed: The terminal body weights and weights of the organ collected (as indicated in the Table 93) were recorded for all animals at study termination at the scheduled necropsy.
Histopathology: Microscopic examination was performed on all tissues listed in Table 91 from all animals in all dose groups.

[1] erythrocytes, hematocrit, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, reticulocytes, thrombocytes, white blood cell (total and differential), fibrinogen, activated partial thromboplastin time, prothrombin time
[2] ALT, AST, AP, γ-glutamyltransferase, total bilirubin, total protein, albumin, globulin, A/G ratio, glucose, urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, cholesterol, triglycerides
[3] microscopic examination, pH, specific gravity, volume, bilirubin, urobilinogen, glucose, ketones, protein.

Toxicokinetics: Blood samples for test substance determination were collected from all non-fasted dogs from a jugular or cephalic vein at 0.5, 1, 2, 4, 8 and 24 hr post dose on study days 1, 91 and 273.

Table 93. Tissues/organs sampled for histopathological examination

Organ/Tissue			Organ/Tissue		
adrenal (2)	W	P,E	mammary glands (females)		P,E
animal identification			muscle, biceps femoris		P,E
aorta		P,E	optic nerve (2)[b]		P,E
brain	W	P,E	ovary (2)	W	P,E
cecum		P,E	pancreas		P,E
cervix[a]	W	P,E	pituitary gland	W	P,E
colon		P,E	prostate	W	P,E
duodenum		P,E	rectum		P,E
epididymis (2)	W	P,E	salivary gland (mandibular [2])		P,E
esophagus		P,E	sciatic nerve		P,E
eye (2)[b]		P,E	skin/subcutis		P,E
femur with bone marrow (articular surface of the distal end)		P,E	spinal cord (cervical, thoracic, and lumbar)		P,E
gall bladder (drained)		P,E	spleen	W	P,E
heart[c]	W	P,E	sternum with bone marrow		P,E
ileum		P,E	stomach		P,E
jejunum		P,E	testis (2)[b]	W	P,E
kidney (2)	W	P,E	thymus	W	P,E
lacrimal glands		P,E	thyroid (2 lobes) with parathyroid	W	P,E
lesions		P,E	tongue		P,E
liver	W	P,E	trachea		P,E
lungs with large bronchi	W	P,E	urinary bladder		P,E
lymph nodes (mandibular)		P,E	uterus[a]	W	P,E
lymph nodes (mesenteric)		P,E	vagina		P,E

E = Examined microscopically; P = Processed; W = Weighed.

a Organs weighed together; uterus with cervix.

b Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

c Weighed after making a cut at the apex to drain the blood from the heart (see Protocol Deviations).

Results

Analysis of Formulations: The achieved concentrations of MYK-461 for all formulations prepared in weeks 7, 8, 13, 21, 29 and 39 were within the ± 15% acceptance criteria of the nominal concentration.

Mortality: All animals survived until their scheduled euthanasia.

Clinical Signs: No test substance-related clinical signs were noted during the dosing or recovery phase.

Body Weights: Test substance did not affect mean body weight or mean body weight gain during the dosing or recovery phase.

Food Consumption: No treatment-related changed were noted for any dose group during the dosing or recovery phase.

Ophthalmoscopy: No remarkable treatment-related ocular changes were noted in any of the groups.

ECG: MYK-461 markedly (P <0.05) prolonged (+16 to +35 msec, 7 to 15%) QTcf interval in animals receiving ≥0.18 mg/kg/day relative to control on day 267 of the dosing phase (Table 94). On an individual animal level, on day 267 of the dosing phase, 3 of 4 males and 3 of
4 females receiving 0.18 mg/kg/day, all high dose males and females demonstrated longer (P <0.05) QTcf intervals than was on day 8 of the predose phase. The prolonged intervals were not observed on day 26 of the recovery phase in 50% of the high dose males and females.

Table 94. Summary of QTcf interval data (msec) in males and females

Group	Dose (mg/kg/day)	Statistics	Pre-dose Phase day 8	Dosing phase day 267 (1 hr post-dose)	Recovery phase day 26	Pre-dose Phase day 8	Dosing phase day 267 (1 hr post-dose)	Recovery phase day 26
			Males			**Females**		
1	0	Mean	236	234	247	245	234	240
		SD	15.6	12.1	11.5	17.8	7.5	17.0
		N	6	6	2	6	6	2
2	0.06	Mean	251	244	-	240	234	-
		SD	6.5	5.2	-	12.9	9.3	-
		N	4	4	-	4	4	-
3	0.18	Mean	246	257* (10%)	-	244	250* (7%)	-
		SD	5.4	15.9	-	15.7	10.9	-
		N	4	4	-	4	4	-
4	0.3 M/ 0.45 F	Mean	242	264* (13%)	232	241	269* (15%)	242
		SD	8.5	9.9	2.8	8.8	10.0	0.5
		N	6	6	2	6	6	2

* P<=0.05. % increase is compared to controls

Mean QRS duration was prolonged (+4 msec, 12%) in females receiving 0.45 mg/kg/day relative to control and predose phase. The prolongation was not observed in

50% of the high dose females on day 26 of the recovery phase. No rhythm abnormalities attributed to test substance administration was observed. A marked increase in heart rate relative to controls was noted in high dose males (+21 bpm, +24%) and in mid (+23 bpm, +29%) and high (+26 bpm, +33%) dose females on day 267 of the dosing phase. A partial reversal in increase in heart rate was noted in two males administered 0.30 mg/kg/day and two females administered 0.45 mg/kg/day on day 26 of the recovery phase.

Echocardiogram Examinations: MYK-461 was associated with progressive and substantial increases in mean left ventricular end-diastolic (LVED) and mean LV end-systolic (LVES) volumes and a substantial ($P < 0.05$) reduction in mean LV ejection fraction in high dose males and females during the dosing phase relative to predose phase (Table 93). The effects were noted by day 76 (Week 11) and progressively increased (more severe) by day 256 (Week 37) of the dosing phase. On day 256 (males and females combined), LVED volume increased by a maximal amount of 67% relative to a growth-related increase of 41% for control animals, while the LVES volume increased by a maximal amount of 220% relative to a 49% increase observed for control animals. Left ventricular ejection fraction (LVEF), an indicator of LV systolic performance, was substantially reduced (25% reduction) by day 76 and progressively reduced (33% reduction) by day 256 in the high dose group animals (male and female combined) relative to predose values (Fig. 44). Control group animals demonstrated 3.2% reduction in LVEF relative to predose values.

The E/A velocity ratio is a marker of the function of the left ventricle of the heart. It represents the ratio of peak velocity flow in early diastole (the E wave) to peak velocity flow in late diastole caused by atrial contraction (the A wave). Abnormalities in the E/A ratio suggest that the left ventricle cannot fill with blood properly in the period between contractions, i.e., diastolic dysfunction. In a healthy heart, the E velocity is greater than the A velocity (>E/A ratio). In diastolic dysfunction, the left ventricular wall is stiff, increasing the back pressure as it fills, which slows the early (E) filling velocity, <E/A ratio. In the high dose group animals, on day 256 of the dosing phase, an increase in E/A ratio and an increase in E-wave deceleration time were noted (Table 95). There were no changes in the isovolumic relaxation time and left atrial dimensions in these animals. No echocardiographic effects were noted for animal receiving 0.06 or 0.18 mg/kg/day. Test substance-related effects developed during weeks 23 and 37 of the dosing phase were reduced during Week 4 of the recovery phase although complete recovery was not documented (Table 95).

Clinical pathology: No consistent effects of treatment on hematology, clinical chemistry or urinalysis parameters. Additionally, MYK-461 administration had no effect on troponin I or NTproBNP test results.

Table 95. Chronic effects of MYK-461 on cardiac function (Echocardiography data)

Dose, mg/kg/day	Control		0.06		0.18		0.3 M	0.45 F
	M	F	M	F	M	F	M	F
Week 11 Dosing phase (n = 4M, 4F evaluated for all dose groups until week 37)								
Left ventricular chamber end-diastolic volume (%)	-	-	-7.4	10.6	4.4	-6.7	10.0	19.6*
LV chamber end-systolic volume (%)	-	-	5.2	9.4	17.2	18.9	70.7*	115.1*
LV ejection fraction (%)	-	-	-5.1	1.1	-4.0	-11.4	-18.4*	-32.6*
Week 23 Dosing phase								
LVEDV (%)	-	-	4.0	11.8	15.1	2.4	22.7*	40.2*
LVESV (%)	-	-	4.3	1.7	0.00	23.7	78.6*	110.2*
LV ejection fraction (%)	-	-	0.3	5.1	5.5	-10.8	-21.0*	-25.5*
Week 37 Dosing phase								
LVEDV (%)	-	-	1.9	14.3*	-2.3	4.0	23.7*	62.9*
LVESV (%)	-	-	35.8	16.3	34.3	30.6	138.8*	202.0*
LV ejection fraction (%)	-	-	-11.9	-0.3	-14.0	-9.6	-32.9*	-33.8*
Transmitral E wave velocities (%)	-	-	-11.1	12.5	0.0	-12.5	-22.2	0.0
Transmitral Deceleration Time (%)	-	-	-22.5	4.5	-14.9	30.9	-34.9	-16.8
Transmitral A wave velocities (%)	-	-	-16.7	50.0	-16.7	25.0	-33.3	25.0
E/A velocity ration	1.6	1.9	0.00	-15.8	0.00	0.0	25.0	-5.3
e' (Mitral annular velocity)	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
E/e' ratio	7.9	9.1	7.6	-9.9	7.6	-19.8	-9.9	11.0
Cmax, ng/ml (see Table 93)					273	300	445	708
NT-proBNP, pmol/ml	745 ± 77				550 ± 76		942 ± 155	
Week 4 Recovery (n = 2M, 2F evaluated for the control and HD groups)								
Left ventricular chamber end-diastolic volume (%)	-	-					-10.0	13.3
Left ventricular chamber end-systolic volume (%)	-	-					-13.9	61.4
LV ejection fraction (%)	-	-					2.3	-20.9
Week 12 Recovery (n = 2M, 2F evaluated for the control and HD groups)								
Left ventricular chamber end-diastolic volume (%)	-	-					4.1	16.1
Left ventricular chamber end-systolic volume (%)	-	-					-9.0	71.7
LV ejection fraction (%)	-	-	-	-	-	-	8.7	-20.3

For treated groups, percent difference from control animal values are shown.
- = no noteworthy findings
* = $p<0.05$ based on actual data (not percent differences).

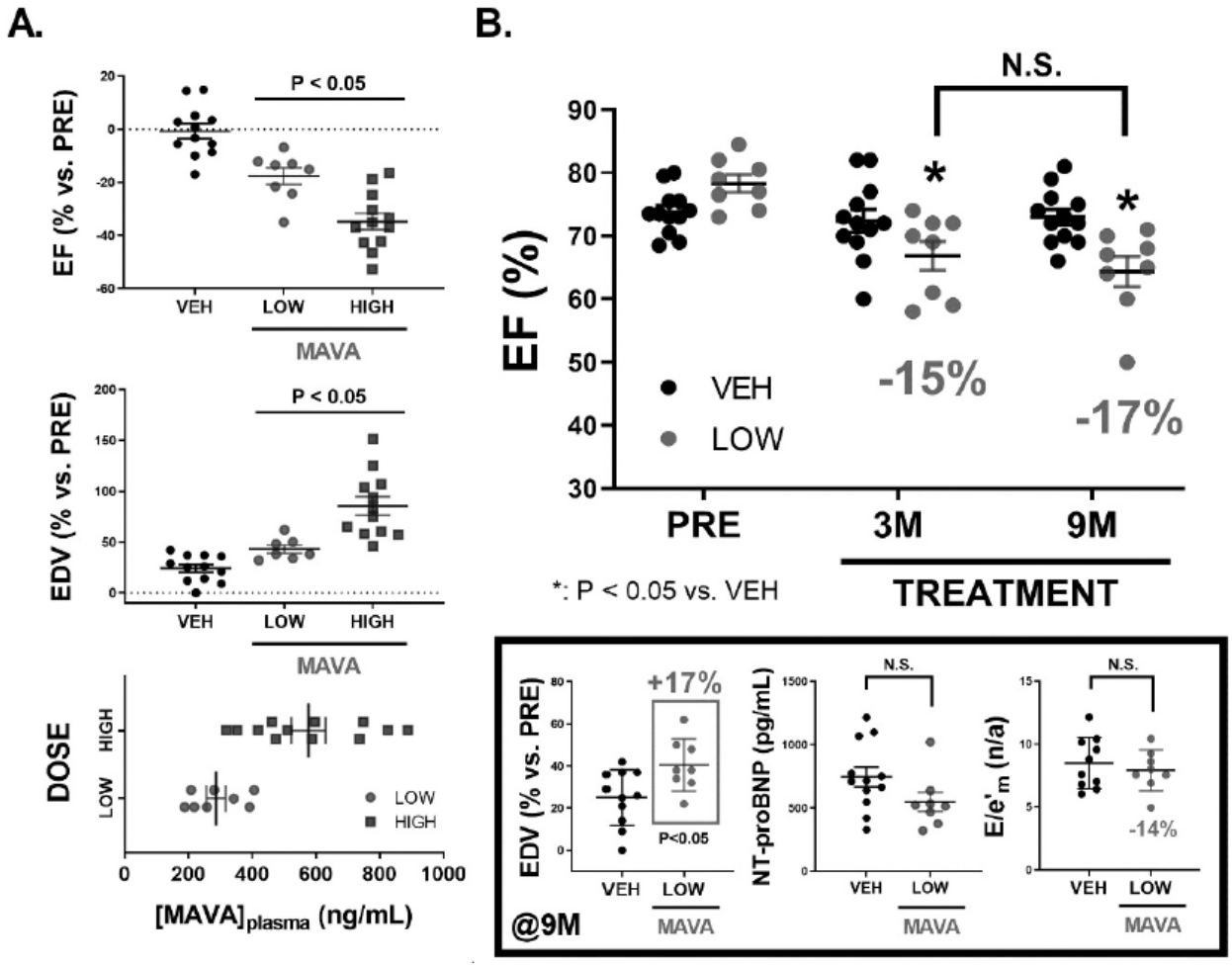


Figure 44. Cardiac effects of MYK-461 in dogs (Echocardiography data). Negative inotropy with preserved diastolic stress
Chronic administration of MYK-461 (MAVA) for up to 9 months (at LOW: 0.18 mg/kg/day or HIGH: 0.3-0.45 mg/kg/day dose levels) triggered sustained exposure-dependent reductions in ejection fraction (EF) with marked preload recruitment (EDV) (see Panel A). Notably, MAVA preserved left atrial dimensions (eLAd), lateral peak early diastolic mitral annulus velocities (e'), circulating NT-proBNP levels, and the E/e' ratio, suggesting preserved filling pressures and improved compliance (see Panel B).

Gross Pathology: No macroscopic changes were noted at the terminal or recovery euthanasians.
Organ Weights: No noteworthy changes were recorded.
Histopathology: No test substance-related microscopic findings were noted at any dose level. Test article-related findings were limited heart effects manifested by electrocardiography changes at ≥0.18 mg/kg/day and echocardiography changes at the high doses of 0.30 (males) or 0.45 (females) mg/kg/day. Thus, the NOAEL is 0.18 mg/kg/day.

Toxicokinetics: The systemic exposure (Cmax and AUC) increased approximately proportionately to the increase in dose in both males and females on all days of measurement. Peak concentration reached at 0.5 hr. There was no sex-dependent difference in the systemic exposure to MYK-461 following administration of 0.06 and 0.18 mg/kg/day. Accumulation (in the range of 2 to 9 for males and 2.6 to 8.3 for females) was noted after repeated daily oral doses. The data suggest that the steady state was not completely achieved on day 273 (week 39), the last day of measurement (Table 96). Interconversion of MYK-461 to its enantiomer, MYK-460, after either single-dose or repeat-dose administration is not discussed in the report.

Table 96. Summary of toxicokinetic parameters of MYK-461 in dogs treated orally with MYK-461

Mavacamten Dose (mg/kg/day)	Study Day	C_{max} (ng/mL) of Mavacamten		AUC_{0-24} (ng*hr/mL) of Mavacamten	
		Male	Female	Male	Female
0.06	1	32	20.2	221	148
	91	69.7	55.5	1100	851
	273	81.5	68.1	1520	1180
0.18	1	93.5	84.9	572	584
	91	188	241	3130	3420
	273	273	300	3130	3420
0.30	1	144	-	924	-
	91	379	-	7490	-
	273	445	-	7960	-
0.45	1	-	295	-	1730
	91	-	758	-	12100
	273	-	708	-	12500

7 Genetic Toxicology

7.1 Ames assay. In vitro bacterial mutation test of MYK-461

Conducting laboratory and location: (b) (4)

Test facility study: # (b) (4) 0370
Sponsor Study: #NC-15-0005
Date of study initiation: May 19, 2014
Date of study completion: May 31, 2014
Drug lot number: MYK-461-6 (Lot 6, doses were expressed as free base)
GLP compliance: Yes
QA statement: Audited, report signed

Key Study Findings

MYK-461 tested up to a maximum concentration of 5000 µg/plate was reproducibly negative for mutagenicity in all tester strains both with and without metabolic activation.

Methods

Test substance was prepared as a stock (50 mg/ml) in DMSO, and dilutions were made from this stock in DMSO. No precipitation was observed. The stability and homogeneity of formulations were not assessed. Duplicate samples were aliquoted from each dose formulation sample for analysis of concentration.

Four Salmonella typhimurium strains and one Escherichia coli strain were used, with or without metabolic activation. The S. typhimurium strains were, TA98, TA100, TA1535, and TA1537; the E. coli strain was WP2uvrA (pKM101). Seven dose levels up to the standard limit of 5000 µg/plate was tested (plate incorporation method, 72 h incubation at 37ºC, 3 plates/dose) with and without metabolic activation. Vehicle and positive controls were also included. The appearance of the background bacterial lawn was examined and revertant colonies counted using an automated colony counter. Toxic effect of the test substance at any dose level was detected by a substantial reduction in mean revertant colony counts, by a sparse or absent background bacterial lawn, or both. No dose range-finding study was performed. One independent assay was performed.

Basis of dose selection: ICH guideline
Metabolic activation system: S-9 homogenate (liver microsomal enzymes) prepared from livers of male Sprague Dawley rats given phenobarbital sodium (30 mg/kg 4 days

before killing and 60 mg/kg 1, 2 and 3 days before killing) and 5,6-benzoflavone (80 mg/kg 2 days before killing) to stimulate mixed-function oxidases in the liver. Procured from a commercial source and stored at -80ºC. A 10% v/v S-9 mix was prepared from the stock.

Controls

Negative control: DMSO

Positive controls: Each tester strain was treated with an appropriate positive control substance (Table 97).

Table 97. Bacterial reverse mutation assay, positive controls

Tester Strain	S9 Mix	Positive controls	Conc. Per plate
TA98	+	Benzo[a]pyrene	5.0 µg
TA98	-	2-nitrofluorene	2.0 µg
TA100	+	2-aminoanthracene	5.0 µg
TA100	-	Sodium azide	2.0 µg
TA1535	+	2-aminoanthracene	5.0 µg
TA1535	-	Sodium azide	2.0 µg
TA 1537	+	Benzo[a]pyrene	5.0 µg
TA 1537	-	9-aminoacridine	50.0 µg
WP2 *uvr*A	+	2-aminoanthracene	10.0 µg
WP2 *uvr*A	-	4-Nitroquinoline N-oxide	2.0 µg

All positive controls were prepared in DMSO.

Criteria for a valid study: The mean number of spontaneous revertants in the vehicle control for each strain should be close to or within the historical control range of the laboratory. All positive controls should produce at least a 2-fold (3-fold for strains TA1535 and TA1537 since they have relatively low spontaneous reversion rates) increase in the number of revertants over the mean value of the respective vehicle control. Mean viable cell counts in the bacterial cultures must be at least 10^9/ml.
Criteria for a positive result: For test substance, the results are considered positive, if there is a substantial increase in revertant colony counts. It should provide an evidence of a dose-dependent increase in mean revertants/plate. For tester strains TA1535, and TA1537, the increase in mean revertants should be three times the mean vehicle control value. For the rest of the tester strains, the increase in mean revertants should be at least 2 times the mean vehicle control value. Treatment-associated increases in revertant colony numbers below 2-3 times those of the vehicle controls are not considered biologically important. Statistical analyses were not performed.

Results

Chemical analyses indicated achieved concentrations within ± 15% of theoretical concentration for test formulations. The sensitivity of the cultures and activity of the S9 mix were demonstrated with substantial increases in revertant colony numbers with all positive control chemicals. The revertant colony counts for the vehicle controls were within or close to the historical control range for the laboratory.

Toxicity, as a reduction in the number of revertant colonies, was observed at 5000 µg/plate in tester strain TA1535 without S9 mix. Precipitate was noted on all plates containing MYK-461 at ≥1500 µg/plate. No positive mutagenic responses were observed with any of the tester strains following exposure to MYK-461 at any of the concentration up to and including 5000 µg/plate in either the presence or absence of S9 activation (Table 98).

Thus, it is concluded that under the conditions of the study, MYK-461 (tested up to a maximum concentration of 5000 µg/plate) provided no evidence of mutagenic activity in either presence or absence of S9 activation.

Table 98. Bacterial reverse mutagenicity data: Summary of test results with MYK-461 obtained in the absence or presence of metabolic activation

Test Article -S9	Concentration (µg/plate)	Revertant Colony Counts (Mean ± Standard deviation) Assay without S9 mix				
		TA98	TA100	TA1535	TA1537	WP2 *uvr*A
DMSO	0	48.7 ± 9.8	160 ± 11.0	20.0 ± 2.0	21.0 ± 1.0	100.0 ± 7.0
MYK-461	5	43.7 ± 12.6	172.3 ± 1.5	13.3 ± 2.5	17.3 ± 8.4	87.7 ± 5.7
	15	40.3 ± 4.7	131.3 ± 11.2	27.0 ± 5.0	20.3 ± 4.7	91.3 ± 3.1
	50	42.0 ± 2.0	150.7 ±4.6	20.0 ± 6.6	19.0 ± 1.7	97.0 ± 7.9
	150	52.7 ± 14.6	153.7 ± 4.5	26.0 ± 4.4	13.0 ± 0.0	105.3 ± 9.8
	500	33.0 ± 1.0	168.0 ± 19.1	27.3 ± 7.1	12.0 ± 4.6	104.7 ± 19.6
	1500	30.7 ± 5.9 P	146.7 ± 23.4 P	32.0 ± 4.4 P	16.3 ± 4.0 P	99.7 ± 13.2 P
	5000	38.0 ± 6.1 P	151.3 ±11.4 P	5.3 ± 2.5 P	12.3 ± 6.0 P	79.3 ± 13.0 P
2NF	2	297.0 ± 87	N/A	N/A	N/A	N/A
NaN3	2	N/A	854.7 ± 113.7	854.7 ± 204.5	N/A	N/A
AAC	50	N/A	N/A	N/A	434.3 ± 140.0	N/A
NQO	2	N/A	N/A	N/A	N/A	1603.3 ± 229

Test Article + S9	Concentration (µg/plate)	Revertant Colony Counts (Mean ± SD). Assay With S9 mix				
		TA98	TA100	TA1535	TA1537	WP2 *uvr*A
DMSO	0	57.3 ± 6.4	161.0 ± 30.4	19.3 ± 5.5	35.0 ± 1.7	86.3 ± 13.2
MYK-461	5	51.7 ± 4.0	136.3 ± 7.4	17.0 ±7.0	31.0 ± 10.6	75.3 ± 13.1
	15	56.0 ±10.5	157.7 ± 10.6	19.3 ± 11.2	37.0 ± 5.2	94.3 ± 10.7
	50	57.3 ± 8.3	148.0 ± 5.6	14.7 ± 3.5	31.3 ± 3.8	100.7 ± 11.7
	150	58.3 ± 3.1	161.0 ± 21.2	22.3 ± 4.0	29.3 ± 6.4	110.7 ± 9.9
	500	64.3 ± 3.8	161.7 ± 11.7	21.0 ± 5.0	27.7 ± 4.2	95.0 ± 19.0
	1500	51.3 ± 8.6 P	159.7 ± 6.5 P	15.0 ± 1.7 P	18.7 ± 1.2 P	130.7 ± 6.0 P
	5000	43.0 ± 4.4 P	164.7 ± 9.5 P	14.0 ± 2.0 P	25.7 ± 4.7 P	105.3 ± 25.6 P
B[a]P	5	302.0 ±11.8	N/A	N/A	N/A	N/A
AAN	5	N/A	2261.0 ± 131	332.7 ± 42.0	N/A	N/A
B[a]P	5	N/A	N/A	N/A	176.7 ± 1.5	N/A
AAN	10	N/A	N/A	N/A	N/A	651.0 ±59.3

P = precipitate; N/A = not applicable; DMSO = dimethyl sulfoxide; 2NF = 2-nitrofluorene; NaN3 = sodium azide; AAC = 9-aminoacridine; NQO = 4-nitroquinoline-1-oxide; B[a]P = benoz[a]pyrene; AAN = 2-aminoanthracine

7.2 Ames assay. In vitro bacterial mutation test of MYK-460 (enantiomer of MYK-461)

Conducting laboratory and location: (b) (4)

Test facility study: #8426936
Sponsor Study #NC-20-0018
Date of study initiation: June 5, 2020
Date of study completion: July 10, 2020
Drug lot number: MYK-460, lot number 400-13-01-59
GLP compliance: Yes
QA statement: Audited, report signed

Key Study Findings

MYK-460 (enantiomer of MYK-461) tested up to a maximum concentration of 5000 µg/plate was reproducibly negative for mutagenicity in all tester strains both with and without metabolic activation.

Methods

Experimental details are similar to those discussed in previous section 7.1 for MYK-461).

For the study, five Salmonella typhimurium strains (TA98, TA100, TA102, TA1535, and TA1537) were used, with or without metabolic activation. Seven dose levels up to the standard limit of 5000 µg/plate was tested (plate incorporation method, 72 hr incubation at 37ºC, 3 plates/dose) with or without metabolic activation. Vehicle and positive controls were also included. No dose range-finding study performed. One independent assay was performed. Negative control was DMSO and each tester strain was treated with an appropriate positive control substance (see Table 97).

Results

Chemical analyses indicated achieved concentrations within 100 ± 10% of the nominal test article concentrations. Toxicity, as a reduction in the number of revertant colonies, was observed at 5000 µg/plate in tester strain TA1535 in the presence of S9 mix. Precipitation was noted on all plates containing MYK-460 at ≥1600 µg/plate.

The numbers of revertant colonies for the vehicle controls were within or close to the historical control range for the laboratory. The positive control chemicals induced increases in revertant numbers of ≥1.5-fold (in strain TA102), ≥2-fold (in strains TA98 and TA100) or ≥3-fold (in strains TA1535 and TA1537) the concurrent vehicle control confirming the validity of the assay.

No positive mutagenic responses were observed with any of the tester strains following exposure to MYK-460 at any of the concentration up to and including 5000 µg/plate in either the presence or absence of S9 activation (Table 99).

Thus, it is concluded that under the conditions of the study, MYK-460, the enantiomer of MYK-461 (tested up to a maximum concentration of 5000 µg/plate) provided no evidence of mutagenic activity in either presence or absence of a rat liver metabolic activation system.

Table 99. Bacterial reverse mutagenicity data: Summary of test results with enantiomer MYK-460 in the absence or presence of metabolic activation

Metabolic Activation	Test Article	Concentration (µg/plate)	Revertant Colony Counts (Mean ± Standard Deviation)				
			TA 1535	TA 1537	TA 98	TA 100	TA 102
Without S9	DMSO	0	9.0±2.6	9.3±5.1	34.3±6.0	94.3±8.6	234.7±15.9
	MYK-460	5	17.0±3.5	9.3±3.5	34.7±3.5	89.3±8.4	220.0±40.4
		16	8.3±1.2	12.0±1.0	33.0±4.6	87.0±3.5	229.0±23.6
		50	8.0±2.6	11.0±1.7	33.7±9.3	96.7±5.5	243.0±2.6
		160	7.7±4.5	10.0±3.6	37.0±3.6	100.7±9.5	227.3±7.8
		500	9.3±3.5	9.3±3.5	37.0±2.0	87.3±6.8	216.3±9.3
		1600	10.7±2.6	6.3±2.9	31.0±1.7	96.7±8.0	208.7±34.0
		5000	11.3±3.2	9.0±3.5	20.7±5.7	82.3±3.5	202.0±40.1
	NaN_3	2	673.7±82.2	NA	NA	811.3±31.8	NA
	AAC	50	NA	383.0±38.6	NA	NA	NA
	2NF	2	NA	NA	1300.3±96.3	NA	NA
	MMC	2	NA	NA	NA	NA	885.0±44.3
With S9	DMSO	0	13.3±2.9	22.7±4.5	54.3±10.8	138.3±6.4	209.0±3.6
	MYK-460	5	15.3±0.6	27.0±5.2	48.3±7.6	129.3±12.0	138.0±1.0
		16	8.0±6.1	27.0±6.6	43.3±2.5	129.3±12.2	238.0±27.2
		50	8.3±3.1	27.7±5.5	48.3±3.1	127.7±3.8	207.3±22.3
		160	8.0±1.0	32.0±5.3	46.0±7.9	145.7±3.2	210.3±15.6
		500	7.3±6.7	31.3±7.0	39.0±7.2	131.3±15.5	178.0±5.6
		1600	8.7±1.5	26.3±4.2	43.7±9.3	131.7±7.6	98.3±35.8
		5000	7.0±1.7	13.3±0.6	36.3±6.0	148.3±15.9	138.7±36.0
	AAN	5	177.7±3.8	364.0±21.0	NA	2822.3±94.6	NA
	AAN	20	NA	NA	NA	NA	2380.7±163.1
	B[a]P	5	NA	NA	385.7±5.0	NA	NA

B[a]P = Benzo[a]pyrene; AAN = 2-Aminoanthracene; 2NF=2-Nitrofluorene; NAN3=Sodium azide; AAC = 9-Aminoacridine; MMC = Mitomycin C; NA=not applicable as the positive control was not utilized for the strain.

7.3. In vitro micronucleus test of MYK-461 in human lymphocytes

The objective of the study was to assess the potential of MYK-461 and its metabolites to induce an increase in the induction of micronuclei in cultured human lymphocytes in vitro.

Key Study Findings

MYK-461 (tested up to a maximum concentration of 100 µg/ml) did not show any clastogenic potential in the chromosomal aberration test with human peripheral blood lymphocytes.

Conducting laboratory and location:	[redacted] (b) (4)
Test facility study:	(b) (4) 0372
Sponsor Study:	NC-15-0004
Date of study initiation:	May 19, 2014
Date of completion:	June 23, 2014
GLP compliance:	Yes
QA statement:	Audited and signed
Drug, lot #, and % purity:	MYK-461, MYK-461-6, 99.9%

Methods

Cell line:	Cultured human peripheral blood lymphocytes prepared from two (pooled equally) healthy non-smoking volunteers
Concentrations in initial test:	3-hr treatment in the absence or presence of S9 mix: 0.53 to 273.3 µg/ml
Concentrations in main test	a) 3-hr treatment in the absence of S9 mix: 10, 190 and 270 µg/ml b) 3-hr treatment in the presence of S9 mix: 100, 190 and 230 µg/ml c) 20-hr treatment in the absence of S9 mix: 5, 30 and 50 µg/ml
Basis of concentration selection:	Cytotoxicity
Negative control:	DMSO
Positive control:	Mitomycin C (MMC), a direct acting mutagen/clastogen, at concentrations of 0.2 and 0.3 µg/ml for 3-h treatment; 0.05 and 0.1 µg/ml for 20-hr treatment. Additionally, colchicine was used at concentrations of 0.05, 0.06 and 0.07 µg/ml for 3-h treatment; 0.01, 0.02 and 0.03 µg/ml for 20-h treatment. Cyclophosphamide (CP), an indirect-acting mutagen/clastogen, at concentrations of 5 and 10 µg/ml. All drugs were prepared in sterile distilled water.
Formulation/Vehicle:	DMSO Stock solution was prepared in DMSO and dilutions were made directly from this solution. Test substance was stable in DMSO. Duplicate samples were collected

	and analyzed for achieved concentrations in the main test.
Activation system:	Male Sprague-Dawley rats dosed with phenobarbital and 5,6-benzoflavone to stimulate mixed-function oxidases in the liver, was purchased from a commercial source
Incubation & sampling time:	All treatments were initiated approximately 48 h following culture stimulation with phytohemagglutinin. Preliminary concentration range test: Test substance preparations were added to cultures (with or without S9 mix) at 1% v/v and were incubated at 37°C for 3 h. At the end of treatment, the cells were centrifuged and replaced with fresh medium. Cultures were treated with cytochalasin B (6 µg/mL, to block cytokinesis) and incubated for an additional 17 h until the scheduled harvest time. For 20-h treatment, test substance was added in the presence of cytochalasin B. S9 mix was not added. Main test: Procedure was similar to the above except that the test included positive control cultures and n = 2 for treatment and n = 4 for vehicle controls.
Harvesting, fixation and slide preparation	The cells were harvested 20 h after treatment initiation, followed by centrifugation for 5 min. Cell pellet washed twice, fixative added, and the cells stored until slide preparation. The incidence of mononucleate, binucleate and polynucleate cells was assessed per culture. The presence of an unusual number of, for example, cells undergoing mitosis, polyploid cells, necrotic cells and debris, if any, was also noted. The incidence of micronucleated cells per 1000 binucleate cells per culture was scored where possible.

In order to assess the cytotoxicity of MYK-461 to cultured human lymphocytes, the cytokinesis- block proliferative index (CBPI) was calculated for cultures treated with test

substance, vehicle and positive controls. The highest concentrations selected were those that caused a reduction in CBPI equivalent to 55 ± 5% cytotoxicity relative to the concurrent vehicle control.

For an assay to be considered valid, the vehicle control group results should show reproducible low and consistent micronucleus frequencies. The positive controls must show clear unequivocal positive responses.

A test article is considered positive if it induces a statistically significant and dose-dependent increase in the frequency of micronucleated cells compared with the solvent control. The increases are reproducible between replicate cultures. The increases are not associated with large changes in pH or extreme toxicity. A negative response is claimed if no statistically significant increases in the number of micronucleated cells above concurrent solvent control frequencies are observed at any concentration, and there is no evidence of a concentration-response relationship.

Results

Chemical analyses indicated achieved concentrations within ± 10% of nominal concentration for all test formulations analyzed.

In the preliminary test, substantial cytotoxicity was observed at concentrations of 273.3 µg/ml (3-hour treatments in the absence or presence of metabolic activation) and 68.8 µg/ml (20-hour treatment in the absence of metabolic activation). In the main test, for the 3-hour non-activated and activated treatments, a reduction in cytokinesis-blocked proliferation index (CBPI) equal to 54.4% and 55.6% cytotoxicity at 270 and 230 µg/ml, respectively, was observed. Similarly, for the 20-hour non-activated treatment, a reduction CBPI equal to 54.7% cytotoxicity was observed at 50 µg/ml MYK-461. In all the 3 main test assays, the mean percentages of binucleated cells containing micronuclei in the MYK-461-treated cultures were not significantly increased relative to the vehicle control value. In all assays, positive control cultures demonstrated a statistically significant increase (17% to 31%) in micronucleated cells relative to the vehicle control value (Table 100).

It is concluded that MYK-461 when tested up to a maximum concentration of 50 to 270 µg/ml (depending on the assay) did not show any clastogenic potential in both the presence and absence of metabolic activation in cultured human lymphocytes under the conditions of the assay.

Table 100. MYK-461. In vitro micronucleus assay in human lymphocyte cells.
Main test: 3-h treatment in the absence or presence of S9 mix, and 20-h in the absence of S9 mix

Metabolic Activation	Test Article	Concentration (µg/mL)	Mean CBPI	Mean Cytotoxicity (%)	Binucleated cells containing micronuclei[a]		
					Mean per 1000 cells	Pairwise p-value[b]	Trend test p-value[c]
3-hours without S9	Vehicle	0	1.64	0	7.8	-	-
	Mavacamten	10	1.65	-2.8	8.5	1.000	-
		190	1.48	25.2	6.0	1.000	0.318
		270	1.29	54.4	9.0	0.585	0.815
	MMC	0.3	1.46	27.0	31.0	<0.001***	-
	COL	0.07	1.45	29.9	18.5	<0.001***	-
3-hour plus S9	Vehicle	0	1.71	0	8.5	-	-
	Mavacamten	100	1.71	0.9	6.5	0.707	-
		190	1.48	33.1	6.0	0.707	0.406
		230	1.32	55.6	8.0	0.707	0.938
	CPA	5.0	1.51	28.4	17.0	0.033*	-
20-hours without S9	Vehicle[a]	0.0	1.78	8.5	-	-	-
	Mavacamten	5	1.73	5.9	7.0	0.532	-
		30	1.52	33.3	7.0	0.532	-
		50	1.35	54.7	9.0	0.532	0.649
	MMC	0.05	1.70	10.4	17.0	<0.001***	-
	COL	0.01	1.69	11.4	17.0	<0.001***	-

a =vehicle control DMSO(1%v/v); b=Pairwise comparisons to control using Williams test for mavacamten and the t test otherwise;
c=/trend test p-values are for the linear contrast including the control group and lower concentrations of the same compound. -no significant findings
* p<0.05; ***p<0.001; CBPI = Cytokinesis block proliferative index; MMC = Mitomycin C; COL = Colchicine; CPA = Cyclophosphamide;
- =no significant findings

7.4. In vitro micronucleus test of enantiomer MYK-460 in human lymphocytes

The objective of the study was to assess the potential of MYK-461 and its metabolites to induce an increase in the induction of micronuclei in cultured human lymphocytes in vitro.

Key Study Findings

MYK-460, the enantiomer of MYK-461 (tested up to a maximum concentration of 100 µg/ml) did not show any clastogenic potential in the chromosomal aberration test with human peripheral blood lymphocytes.

Conducting laboratory and location:	[redacted] (b) (4)
Test facility study:	8426937
Sponsor Study:	NC-20-0019
Date of study initiation:	June 5, 2020
Date of completion:	August 5, 2020
GLP compliance:	Yes
QA statement:	Audited and signed
Drug, lot #, and % purity:	MYK-460, 400-13-01-59, 99.9%

Methods

Experimental details are similar to those discussed in previous section 7.3 for MYK-461.

Cell line:	Cultured human peripheral blood lymphocytes prepared from three (pooled equally) healthy non-smoking volunteers
Final concentrations in range-finder expt.:	3-h treatment in the absence or presence of S9 mix: 0.992 to 273.3 µg/ml
Final concentrations in main expt.:	a) 3-h treatment in the absence of S9 mix: 60 to 273.3 µg/ml b) 3-h treatment in the presence of S9 mix: 30 to 273.3 µg/ml c) 20-h treatment in the absence of S9 mix: 5 to 273.3 µg/ml
Basis of concentration selection:	Cytotoxicity
Negative control:	DMSO
Positive control:	Mitomycin C (MMC), a direct acting mutagen/clastogen, at concentrations of 0.05 to 0.4 µg/ml for 3- and 20-h treatments; Cyclophosphamide (CP), an indirect-acting mutagen/clastogen, at concentrations of 2 to 6 µg/ml for 20-h treatment. All drugs were prepared in sterile distilled water.
Formulation/Vehicle:	DMSO Stock solution was prepared in DMSO and dilutions were made directly from this solution. MYK-460 was stable in DMSO. Solutions were used within 4 h of preparation. Triplicate samples were

	collected and analyzed for achieved concentrations in the main test.
Activation system:	S-9 liver fraction prepared from male Sprague-Dawley rats treated with Aroclor 1254, was purchased from a commercial source.
Incubation & sampling time:	All treatments were initiated approximately 48 h following culture stimulation with phytohemagglutinin. Preliminary concentration range test: Test substance preparations were added to cultures at 1% v/v and were incubated at 37°C for 3 (with or without S9 mix, +17 h recovery) or 20 (without S9, 0 h recovery) hr. Two hours prior to harvest, colchicine was added to arrest dividing cells in metaphase. At the defined sampling time cultures were centrifuged for 10 min; the supernatant was removed, and cells were resuspended in hypotonic (0.075 M) KCl and incubated at approximately 37°C for 15 min to allow cell swelling to occur. Cells were centrifuged, resuspended and slides were prepared, examined and the Mitotic Index (MI) was determined. Based on the toxicity data, a suitable range of concentrations was selected for the main test. Main test: Concentrations of test substance were selected based on mitotic inhibition. Procedure was similar to the above except that the test included positive control cultures.
Harvesting, fixation and slide preparation	See section 7.3. A minimum of 300 metaphases per concentration (150 metaphases from each code) were analyzed for chromosome aberrations.

Results

Chemical analyses indicated achieved concentrations within 100 ± 10% of nominal concentration for all test formulations analyzed.

In the cytotoxicity range-finder experiment, precipitation of test substance was noted at 164 µg/ml and substantial cytotoxicity (>50%) was observed at concentrations of 35.42 µg/ml in 20-h treatment in the absence of metabolic activation and 273 µg/ml at 3-h treatment (in the absence or presence of metabolic activation). The highest concentration selected for the main experiment did not exceed 50% mitotic inhibition.

In the main test, for the 3-hour non-activated and activated treatments, and 20-hr in the absence of S-9 treatment frequencies of cells with structural chromosome aberrations were similar to and not significantly ($P \leq 0.05$) higher than those observed for the concurrent vehicle control cultures for all concentrations analyzed. The frequencies of cells with structural chromosome aberrations (excluding gaps) also fell within the normal ranges. However, a small, statistically significant linear trend ($P \leq 0.05$) was noted following the 3- + 17-hour treatment in the presence of S-9 (Table 101). According to the sponsor, the statistically significant linear trend is considered of no biological relevance as both individual and mean frequencies of structural chromosome aberrations (including or excluding gaps) of all concentrations analyzed for the 3 + 17-hour treatment in the presence of S-9 fell within the historical normal range (but not concurrent control) (see footnote to Table 101). Furthermore, no increase in frequency of endo-reduplicated and/or polyploid cells was observed relative to the concurrent vehicle control cultures and normal ranges, for all three treatment conditions. The maximum concentrations analyzed were limited by precipitation observed at the end of treatment period (both 3- + 17-hour treatments) or cytotoxicity (for 20- + 0-hour treatment),

In all assays, positive control cultures demonstrated a statistically significant increase (17% to 31%) in micronucleated cells relative to the vehicle control value (Table 101).

Based on the above results, it is concluded that MYK-460, the enantiomer of MYK-461 when tested up to a maximum concentration of 10 to 220 µg/ml (depending on the assay) did not show any clastogenic potential in both the presence and absence of metabolic activation in cultured human lymphocytes under the conditions of the assay.

Table 101. MYK-460. In vitro micronucleus assay in human lymphocyte cells.
Main test: 3-h treatment in the absence or presence of S9 mix, and 20-hour in the absence of S9 mix

Metabolic Activation	Test Article	Concentration (µg/mL)	% Cells with Chromosome Aberrations (Excluding Gaps)	Mean Cytotoxicity (%)	p-value
3+17 hours without S9	Vehicle	0	0.67	0	-
	Mavacamten	120	0.33	2	NS
		160	0.00	22	NS
		220	0.00	42	NS
	MMC*	0.4	35.5	49	≤0.001
3+17 hours plus S9	Vehicle	0	0.33	0	-
	Mavacamten	60	0.33	3	NS
		120	1.00	16	NS
		160	1.33	20	NS
	CPA*	2.0	27.6	39	≤0.001
20+0 hours without S9	Vehicle[a]	0.0	0.33	0	-
	Mavacamten	10	1.00	15	NS
		25	0.33	6	NS
		40	1.00	23	NS
		80	1.67	50	NS
	MMC*	0.1	25.75	44	≤0.001

* positive control; MMC = Mitomycin C; CPA = Cyclophosphamide; - NS=no significant findings

Note: Historical vehicle control range: 3- + 17-hour treatment in the presence of S-9 ranged from 0 to 4.67 (mean 0.86) and 0 to 4 (mean 0.58) for structural aberration including gaps and excluding gaps, respectively. Thus, the statistically significant linear trend noted with MYK-460 is considered of no biological relevance (see text).

7.5. *In vivo* clastogenicity (micronucleus) assay of MYK-461 in rats

The objective of the study was to assess the potential of MYK-461 to induce an increase in the induction of micronucleated polychromatic erythrocytes (a measure of chromosomal damage) in an in vivo cytogenetic test system.

Key Study Finding

MYK-461 was negative causing an increase in the induction of micronucleated polychromatic erythrocytes or bone marrow cell toxicity in male rats when administered orally by gavage.

Conducting laboratory and location:	(b) (4)
Test facility study no.:	14-2420
Sponsor ref. no.:	NC-14-0063
Date of study initiation:	May 5, 2014
Date of completion:	May 23, 2014
GLP compliance:	Yes
QA statement:	Audited and signed
Drug, lot #, and % purity:	MYK-461, 6, 99.79% (MYK-460: 0.21%)

Methods

Dose:	See Table 102
Frequency of dosing:	Single oral gavage for 2 days
Sample time:	All animals were sampled 24 h after the final dose administration (Day 3)
Route of administration:	Orally by gavage,
Dose volume:	5 ml/kg for test substance and vehicle control
Formulation/Vehicle:	Dosing formulations of MYK-461 were prepared in 0.5% (w/v) methylcellulose in distilled water and used within 3 h following preparation. Homogeneity, stability and concentration analyses were performed on dosing formulations prepared on the day of dose preparation.
Species/Strain:	Sprague-Dawley male rats (b) (4)
Number/Group:	See Table 100
Age:	8 weeks old at first dosing
Weight:	233 to 267 gm at dosing
Housing:	Group housed (2-3/cage), *ad libitum* food and water.
Basis of dose selection:	Doses were selected based on a preliminary study in which 2 daily doses of 10 mg/kg/day was administered to 3 male rats. One animal was found dead on day 3. The next dose was reduced to 8 mg/kg/day and was well tolerated.
Negative control:	0.5% (w/v) methylcellulose in water

Positive control: Cyclophosphamide. It was not part of the concurrent assay. Positive control slides were prepared in study (b) (4) S0923, from the same lab.

Main test Animals were not fasted prior to treatment. Two oral gavage doses (separated by 24 h interval) of 1, 3 or 8 mg MYK-461/kg/day or vehicle were administered to each animal (Table 102).

Table 102. Study design - main test

Group	Treatment	Dose (mg/kg/day)[a]	Total on Study Males	Number of animals Micronucleus Assay[b] Males	Satellite study[c] Males
1	Control	0	7	7	0
2	MYK-461	1	10	7	3
3	MYK-461	3	10	7	3
4	MYK-461	8	10	7	3

[a] Doses represent (b) (4) active ingredient.
[b] Terminal necropsy occurred 24 hours (± 1 hour) after the 2nd dose.
[c] Toxicokinetic samples were collected on Days 1 and 2.

Animals were observed for clinical signs of toxicity prior to dose administration and twice daily for mortality and signs for severe toxic or pharmacologic effects until sacrifice. Animals were weighed prior to first dose administration. Blood samples for toxicokinetic determination were collected on days 1 and 2 from 3 animals/groups at 1, 2 and 4 h post dose.

All surviving animals were euthanized 24 h after the 2nd dose. Animals were not fasted. The bone marrows were collected from all animals. Animals were discarded without further evaluation. The content of left femur from each animal was removed, harvested and smears were prepared from each animal. For each animal, six unstained slides were prepared. At least 2000 polychromatic erythrocytes (PCEs) per animal were scored for the presence of micronucleated PCE. The proportion of PCE (as a sign of bone marrow toxicity) was assessed by examination of at least 1000 erythrocytes per animal. In addition, the number of micronucleated normochromatic erythrocytes was recorded. Additionally, 5 slides [prepared in a separate study (b) (4) Study Number (b) (4) S0923)] from animals treated with cyclophosphamide, were stained, blindly coded along with the bone marrow smears prepared from this study and used as the positive control.

For a valid test, vehicle control values for micronucleated PCEs must be consistent with the laboratory historical vehicle control data. The positive control must show a clear unequivocal positive response. The test article was considered positive when group mean incidences of micronucleated PCE exceed (P <0.05) the laboratory historical control range. Bone marrow cell toxicity is normally indicated by a substantial and statistically significant decrease in the proportion of PCEs.

Results

All study samples analyzed achieved mean concentrations within ± 1% of nominal concentrations. No adverse test substance-related clinical signs were noted in the main test animals. In toxicokinetic animals, MYK-461 plasma concentrations increased in an approximately dose-proportional manner over the dose range tested. No statistically significant increases in the frequency of micronucleated PCE and no statistically significant decreases in the proportion of PCE were noted following the administration of 2 doses of MYK-461 relative to vehicle control values. The positive control, CP, induced a substantial increase in micronucleated PCE (slides prepared from a different study and not part of concurrent study) (Table 103).

Table 103. Main study: Group mean summary of micronucleated PCE and proportion of PCE for rats treated with varying doses of MYK-461

Sampling time after 2nd dose	Treatment	Dose (mg/kg/day)	Proportion of PCE (Group mean %) #	Incidence MPCE (Group mean) #
24 Hours	Vehicle	-	55.3	1.9
	MYK-461	1	47.7	1.4
	MYK-461	3	55.8	1.6
	MYK-461	8	53.1	1.6
	Cyclophosphamide [a]	20	42.9*	48.8**

N =7 for vehicle and MYK-461; n =5 for cyclophosphamide.
Vehicle: 0.5% Methylcellulose (MC) in distilled water (USP)
PCE: Polychromatic erythrocytes
MPCE: Number of micronucleated polychromatic erythrocytes observed per 2000 polychromatic erythrocytes examined
a: Positive control slides from (b) (4) 0923
#: Occasional apparent errors 1% may occur due to rounding of values for presentation in the table
Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities): * p < 0.05 (significant), ** p < 0.01 (significant) Otherwise, p > 0.05 (not significant)

8 Carcinogenicity

8.1 Six-month oral gavage carcinogenicity study of MYK-461 in RasH2 transgenic mouse

Conducting laboratory and location: (b) (4)

Test facility study number: NC-18-0002
Study number: 8383637
Study initiation date: September 28, 2018
In-life end date: April 12, 2019
Drug lot/batch number: MYK-461,
GLP compliance: Yes
QA statement: Yes
CAC Concurrence: Yes

Key Study Findings

Oral gavage administration of 0.5, 1, or 2 mg/kg/day MYK-461 to male or 0.5, 1, or 3 mg/kg/day MYK-461 to female RasH2 transgenic mice for 26 weeks was clinically well tolerated, had no effect on survival, and exhibited no evidence of any carcinogenic potential. All neoplastic and non-neoplastic microscopic findings were considered spontaneous and/or incidental because they occurred at a low incidence, the incidence and/or severity of the findings were not dose related, and/or they were of the types expected for RasH2 mice. Positive control mice were diagnosed with malignant lymphoma, squamous cell papilloma and carcinoma in the forestomach, and hemangio-tumors. Based on mean Cmax and AUC values, the systemic exposure at the dose of 2 (male) or 3 (female) mg/kg/day in the Hras2 transgenic mouse exceeds the human Cmax and AUC (10 daily oral doses of 15 mg MYK-461 (MRHD in Explorer MYK-461-005) by a factor of 2.3 and 1.8 for males and 4.4 and 3.0 for females, respectively.

Purpose

The study was conducted to assess the potential toxicity and carcinogenic effects of MYK-461 when administered orally by gavage to RasH2 transgenic hemizygous mice for 26 weeks, and to determine toxicokinetics of MYK-461 when administered daily orally to RasH2 wild type mice.

Methods

Formulation

Test substance was dissolved in 0.5% (w/v) methylcellulose prepared in reverse osmosis water. Drug formulations were prepared every two weeks and stored in a refrigerator. The dosing formulations prepared for administering on Day 1 and during Weeks 13 and 26 of the dosing phase were evaluated for homogeneity and concentration. The positive control article, N-methyl-N-nitrosourea (MNU) was prepared in acidified physiological saline (150 mM sodium chloride and 15 mM sodium citrate in reverse osmosis water adjusted to pH 4.5 ± 0.1 with 1 N hydrochloric acid) once and used within 3 days of preparation. One set of triplicate samples from the positive control article formulations was analyzed for test article content.

Animals

Species/Strain: Male and female RasH2 (001178-T [hemizygous], CByB6F1-Tg[HRAS]2Jic) mice (n = 116 each sex); and male and female RasH2 (001178-W [wild type], CByB6F1-Tg[HRAS]2Jic) mice (n = 63 each sex) were received from (b) (4).

#/Sex/Group: 25 in the control and dose groups; an additional 18 mice (6 for the control) were designated for toxicokinetic; 10 in the positive group (Table 104).

Age: 9 to 11 weeks at start of dosing

Weight: Males: 21.1 to 35.1 g; Females: 18.0 to 26.6 g at the start of dosing

Husbandry: Males were individually housed, while females were group-housed (up to three mice/cage) except during study-related procedures. Certified diet and water were provided ad libitum.

Dosing

Doses: Male and female animals were randomly assigned to five dose groups (Table 104).

Mode and Duration of Administration: MYK-461 was administered once daily orally by gavage at dose levels of 0.5, 1, or 2 mg/kg/day for males, and 0.5, 1, or 3 mg/kg/day for females for up to 26 weeks (182 days). Initially, the sponsor has performed a dose range-finding study at dose levels of 2, 4 or 6 mg/kg/day for 4-week in wild type RasH2 mice. The high dose (6 mg/kg/day) was not tolerated resulting in deaths from heart failure with dilatation and hypertrophy of the heart. There were no deaths/euthanasia at 5 mg/kg/day. A significant ($P < 0.05$) decrease in body weight gain relative to control was noted at all doses for males for days 1 through 28. The NOAEL was 2 mg/kg/day for female mice and there was no NOAEL for male mice (see section 6.2.2 for details). After reviewing the sponsor's study data, the Executive CAC (see Appendix D, meeting minutes of June 14, 2018) recommended doses of 0 (vehicle), 0.5, 1, and 2 mg/kg/day for males, and dose levels of 0.5, 1, and 3 mg/kg/day for females. The sponsor accepted the proposed doses. Control animals (Group 1) received drug vehicle

(10 ml/kg). The positive control group (#5) animals received a single intraperitoneal dose of 75 mg/kg N-methyl-N-nitrosourea (MNU) on Day 1 of the dosing phase. The positive control animals were included so as to appropriately express oncogenes and respond to carcinogenic insult.

Table 104. Study design

Group	Subgroup	No. of Animals		Dose Level (mg/kg/day)		Dose Concentration (mg/mL)[a]	
		Male	Female	Male	Female	Male	Female
1 (Vehicle Control)[b]	1 (Carcinogenicity)	25	25	0	0	0	0
	2 (Toxicokinetic)	6	6	0	0	0	0
2 (Low)	1 (Carcinogenicity)	25	25	0.5	0.5	0.05	0.05
	2 (Toxicokinetic)	18	18	0.5	0.5	0.05	0.05
3 (Mid)	1 (Carcinogenicity)	25	25	1	1	0.1	0.1
	2 (Toxicokinetic)	18	18	1	1	0.1	0.1
4 (High)	1 (Carcinogenicity)	25	25	2	3	0.2	0.3
	2 (Toxicokinetic)	18	18	2	3	0.2	0.3
5 (Positive Control)[c]	1 (Carcinogenicity)	10	10	75	75	7.5	7.5

a Concentrations (Groups 2 through 4 only) were based on test article as supplied. A correction factor was not used.
b Group 1 was administered vehicle control article only.
c Group 5 was dosed with one intraperitoneal dose of N-methyl-N-nitrosourea (MNU) on Day 1 of the dosing phase. These animals were included as positive controls to ensure animals supplied appropriately expressed oncogenes and responded to carcinogenic insult.

Observations and Measurements

Clinical Signs: All animals were observed twice daily for mortality and clinical signs. Detailed observations were made prior to dosing on Day 1, weekly to Week 26 of the dosing phase. Macroscopically visible or palpable mass was recorded.

Body Weights: It was recorded for both carcinogenicity and toxicokinetic animals once during the predose phase, before dosing on Day 1, weekly to Week 26 of the dosing phase, and on Day 182 of the dosing phase.

Food Consumption: Food intake was recorded weekly from Day 1 to Week 26 and from Days 176 to 182 of the dosing phase.

Clinical Pathology: Blood smears were prepared for each euthanized animal during the necropsy procedure on the days of scheduled and unscheduled euthanasia. Bone marrow smears (two slides) were prepared from the femur of each animal at scheduled euthanasia.

Gross Pathology: All surviving animals were fasted overnight and weighed prior to scheduled necropsy on Day 183. A complete necropsy including macroscopic examination of abnormalities and collection of tissues from an extensive list (Table 105) was done on all animals including unscheduled decedents.

Table 105. Tissues/organs sampled for histopathologic examination

Organ/Tissue		Organ/Tissue	
adrenal (2)	P,E	muscle, gastrocnemius	P,E
animal identification	P,E	muscle, quadriceps femoris (cranial thigh)	P,E
aorta	P,E	muscle, soleus (2)	P,E
bone, femur with bone marrow (articular surface of the distal end to include stifle joint)	P,E	nasal turbinates[c]	P,E
bone, sternum with bone marrow	P,E	optic nerve (2)[a]	P,E
brain	P,E	ovary (2)	P,E
cecum	P,E	oviduct (2)	P,E
cervix	P,E	pancreas	P,E
colon	P,E	pituitary gland	P,E
clitoral gland (2)	P,E	preputial gland (2)	P,E
coagulating glad (intact with seminal vesicles [2])	P,E	prostate	P,E
duodenum	P,E	rectum	P,E
epididymis (2)	P,E	salivary gland (mandibular [2])	P,E
esophagus	P,E	salivary gland (sublingual [2])	P,E
eye (2)[a]	P,E	salivary gland (parotid [2])	P,E
gall bladder (drained)	P,E	sciatic nerve (see Protocol Deviations)	P,E
gut-associated lymphoid tissue (GALT)/Peyer's patch	P,E	seminal vesicle	P,E
Harderian gland (2)	P,E	skin/subcutis	P,E
heart[b]	P,E	spinal cord (cervical, thoracic, and lumbar)	P,E
ileum	P,E	spleen	P,E
jejunum	P,E	stomach	P,E
kidney (2)	P,E	testis (2)[a]	P,E
lacrimal gland (2)	P,E		
larynx	P,E	thymus	P,E
lesions	P,E	thyroid (2 lobes) with parathyroid	P,E
liver	P,E	tongue	P,E
lungs with large bronchi	P,E	trachea	P,E
lymph nodes (mandibular [2])	P,E	ureter (2)	P,E
lymph nodes (mesenteric)	P,E	urinary bladder	P,E
mammary glands (females)	P,E	uterus (body and horns)	P,E
muscle, biceps femoris	P,E	vagina	P,E
muscle, diaphragm	P,E	Zymbal's gland (collect with skull/nasal cavity)[d]	P,E

E = Examined microscopically; P = Processed.

a Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

b The entire heart was immersed into ice-cold 10% neutral-buffered formalin that was stored overnight in a refrigerator (set to maintain a temperature range of 2 to 8°C) and maintained on wet ice once removed from the refrigerator, gently agitated, then refrigerated for at least 24 hours. In the event that an animal died or was euthanized at an unscheduled interval, the requirement to refrigerate the 10% neutral-buffered formalin overnight did not apply.

c Microscopic evaluation of level III.

d Held in fixative.

Organs Weighed: The terminal body weights and weights of the organ collected (adrenal, brain, heart, epididymides, prostate, kidneys, liver, pituitary, ovaries, uterus, spleen, testes, thymus and thyroid) were recorded for all surviving animals at study termination at the scheduled necropsy.
Histopathology: Microscopic examination was performed on all tissues listed in Table 105 from all animals in dose groups 1 to 4 and carcinogenicity and toxicokinetic animals that died or were euthanized at an unscheduled interval. Also, macroscopic lesions and thymus from Group 5 were examined microscopically.
Toxicokinetics: Blood samples for test substance determination were collected from 3 animals/sex/group/time point at 0.5, 1, 2, 6 and 24 h post dose on study days 28 and 182 from Group 2 through Group 4. Blood samples from control animals were collected at 2 and 24 h post dose. The animals were not fasted prior to blood collection. Toxicokinetic animals were euthanized with isoflurane inhalation and discarded without necropsy after the final blood collection. A necropsy was conducted on toxicokinetic animals that died or were euthanized at an unscheduled interval

Results

Analysis of Formulations: The achieved concentrations of MYK-461 for all doses were in the range from 84.7 to 106% of nominal. Also, all formulations met the specifications for homogeneity, with relative standard deviations of between 0.6 and 6.8%. The MNU formulation prepared on Day 1 of the dosing phase ranged from mean concentration of 101.1 to 103.8%.
Mortality: Following dosing of MYK-461 for 26 weeks, the survival rates were 100, 96 (24/25), 92 (23/25), and 100% for the males and 92 (23/25), 96 (24/25), 96 and 96% for the females in the 0, 0.5, 1.0, and 2.0/3.0 mg/kg/day, respectively (Table 106).
The survival rates in the treated groups were comparable to vehicle controls of 100% for males and 92% for females. There were 8 early decedents including 2 control females during the treatment period. Tests to compare survival were performed, with a two-sided risk for increasing and decreasing deaths with dose. Tests were performed for dose response and for each test substance-treated group against control using Kaplan-Meier product-limit estimation curves, along with log-rank and Wilcoxon tests (Table 107). Drug treatment had no effect on mean survival time, survival rate or cause of death. The main cause of moribund sacrifice or death was hemangiosarcoma, malignant lymphoma, hemo-lymphoreticular system in both males and females. No increase over the control incidence of moribund sacrifices or deaths to tumors or toxicity was noted for MYK-461-treated groups. Administration of test substance did not induce early development of spontaneous neoplastic lesions in RasH2 mice.

Survival in the MNU treated positive control animals at the end of the dosing phase was 9% (1/10) for males and 20% (2/10) for females. An increased early death rate in the positive control animals was attributed primarily to the presence of malignant neoplasms, which included malignant lymphoma in multiple tissues, squamous cell carcinoma in the skin/subcutis and nonglandular stomach, and choriocarcinoma in the ovary.

Table 106. Mortality -Survival and incidence and cause of early decedents

Gp	Dose	N	% survival	Animal #	Day of death/ euthanasia	Mortality information
1M	Control	25	100	No early decedents		
1F		25	92	M506 M522	151 E 174 D	Hemangiosarcoma, skin/subcutis Undetermined
2M	0.5	25	96	M125	127 E	Malignant lymphoma, hemo-lymphoreticular system
2F		25	96	M618	148 E	Hemangiosarcoma, vagina
3M	1	25	92	M213 M207	142 D 181 D	Hemangiosarcoma, spleen Sarcoma NOS, prostate
3F		25	96	M720	71 E	Carcinoma, bronchiolo-alveolar, lung
4M	2	25	100	No early decedents		
4F	3	25	96	M810	29 D	Hemangiosarcoma, spleen
5M	Positive control, MNU	10	9[a]	7 animals were moribund sacrificed on Day 15, 2 animals were found dead on Days 90 and 160. Higher death rate was attributed to the presence of malignant neoplasms.		
5F		10	20	2 animals were found dead on Days 73 and 85, 6 animals were moribund sacrificed on Day 73. Higher death rate was attributed to the presence of malignant neoplasms.		

a: Number of animals in group adjusted for accidental deaths.
D: Found dead, E: Euthanasia, moribund sacrifice, F: female, M: Male, NOS: Not otherwise specified

Table 107. Results of Statistical Analysis of Survival Data – Males (left panel), Females (right panel)

		Unadjusted Survival Incidence Rate			
Group	Trend	1	2	3	4
Dose Level(mg/kg/day)	(1,2,3,4)	0	0.5	1	2
Group Size		25	25	25	25
Terminal Sacrifice		25	24	23	25
Deaths		0	1	2	0
Log-Rank P-value (v1)	0.9187	N/A	0.3173	0.1531	N/A
Wilcoxon P-value (v1)	0.9156	N/A	0.3173	0.1531	N/A

	Unadjusted Survival Incidence Rate			
Trend	1	2	3	4
(1,2,3,4)	0	0.5	1	3
	25	25	25	25
	23	24	24	24
	2	1	1	1
0.6726	N/A	0.5610	0.5717	0.5717
0.6906	N/A	0.5717	0.5878	0.5878

Clinical Signs: Abnormal clinical and mass observations in drug-treated animals were observed in animals that died or were moribund sacrificed. These included hunched appearance, low carriage, irregular respiration, pale appearance, piloerection, limited use of limb, mass (axilla), thin, and swollen vagina. Clinical signs noted in terminal sacrificed animals included malocclusion, bent tail, pale body, missing ear, missing teeth, thin, swollen testes, thinning haircoat, and alopecia. These signs were also noted in comparable incidence in controls, and furthermore the lack of dose response, the sponsor considered these findings not MYK-461-related. No palpable masses were observed in drug-treated animals. MNU-treated animals that met early deaths or euthanized in moribund demonstrated hunched posture, low carriage, malocclusion, oral mucosa mass, thin appearance, hypoactive, irregular or labored respiration, cold to touch, pale, and piloerection. Also, these animals had multiple masses consistent with the known carcinogenic activity of MNU. Sporadic observations not associated with an early death or euthanasia were limited to scabs and thinning haircoat.
Body Weights: No decrease in body weight or weight changes relative to control was noted at all doses for both males (Fig. 45) and females (Fig. 46) (for data, see Appendix A). Irregular week-to-week differences in body weight from controls were observed for MYK-461-treated animals, but the differences lacked dose response, and these were considered not treatment-related.

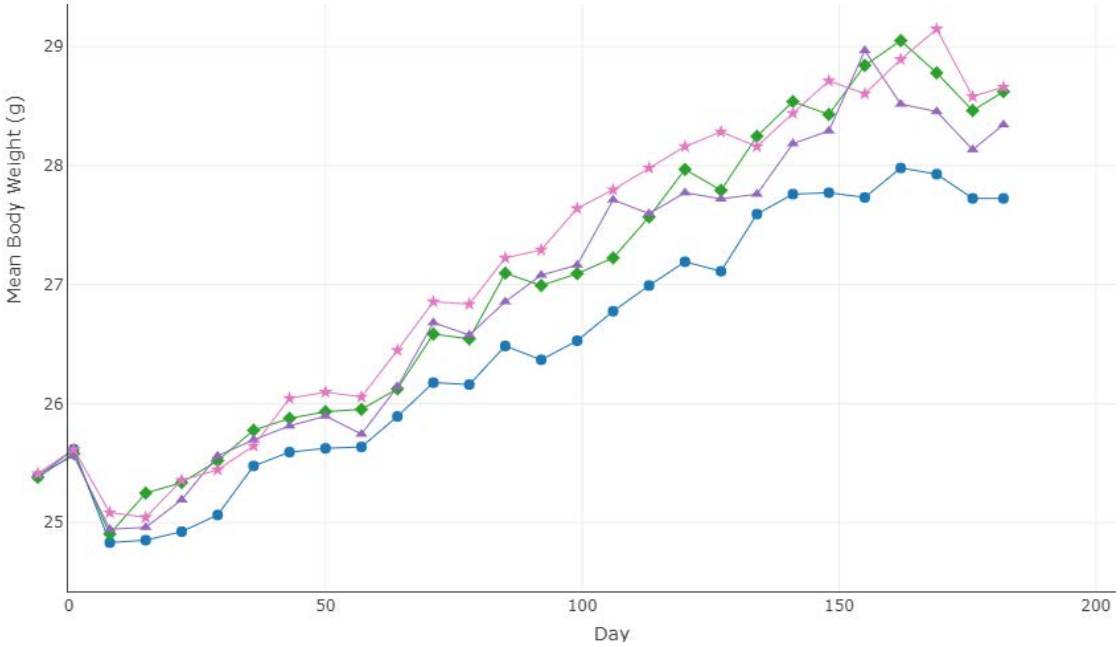


Figure 45. Mean Body Weight Data – Males. For legend, see below

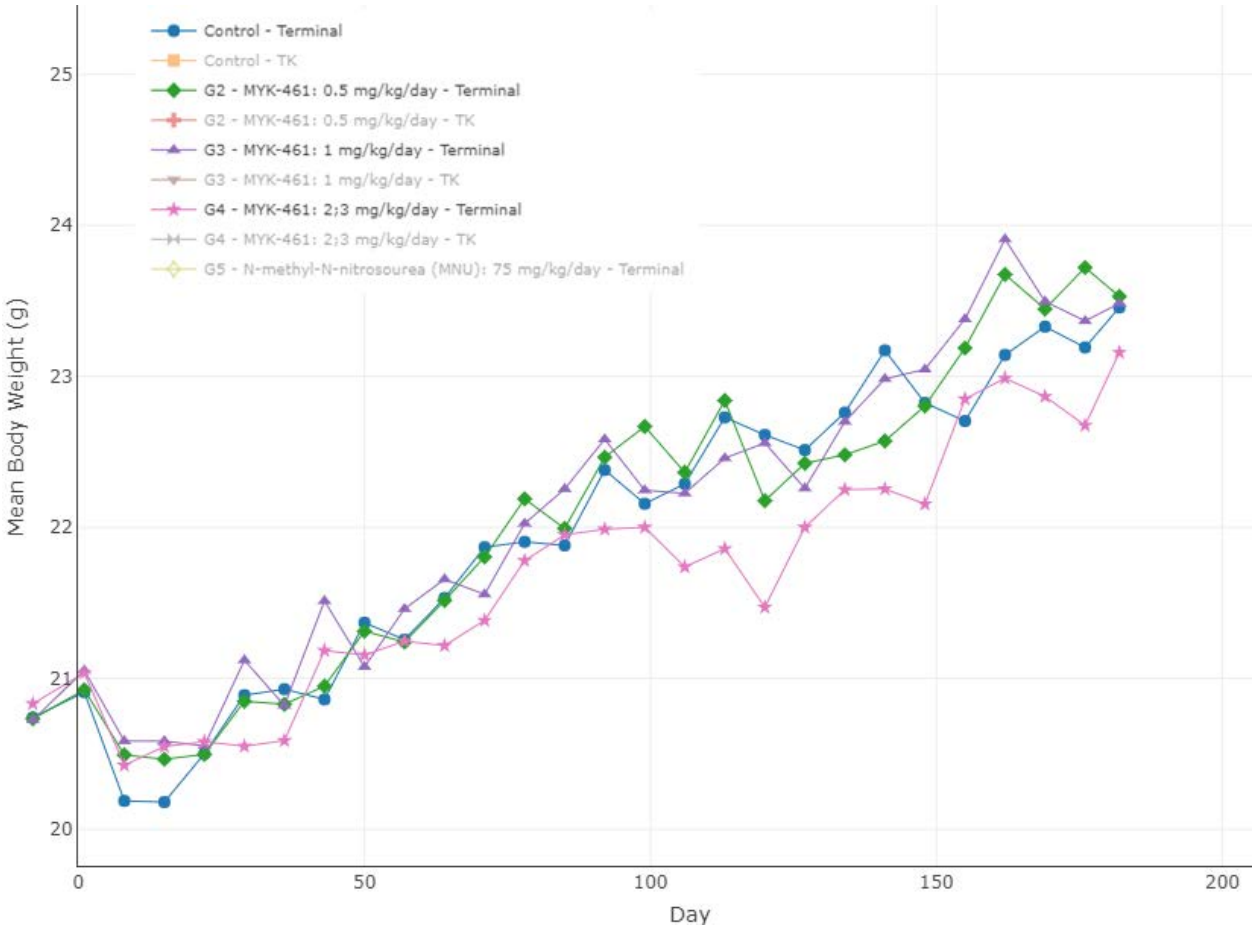


Figure 46. Mean Body Weight Data - Females

Food Consumption: No changes in food consumption were noted.
Organ Weights: Organ weights were recorded at the scheduled euthanasia, but are not included in the study report.
Gross Pathology: No macroscopic findings were noted in animals examined at unscheduled necropsies or the terminal euthanasia. All macroscopic findings were considered spontaneous and/or incidental because they occurred at a low incidence, were randomly distributed across groups (including vehicle controls), and/or were as expected for this mouse strain. Thus, the sponsor considered these findings not related to the administration of MYK-461. Macroscopic findings related to the administration of MNU generally correlated with neoplasms. The correlative microscopic findings in one or more animals included malignant lymphoma (which correlated with enlarged thymus, spleen, liver, kidney, and/or one or more lymph nodes; mass in the thymus and/or ovary; raised area in the liver; rough surface in the kidney; and/or discoloration of the entire spleen), squamous cell papilloma and/or carcinoma (which correlated with

mass and/or raised area of the stomach, skin/subcutis, and/or oral mucosa), and choriocarcinoma of the ovary (which correlated with ovary mass).
Histopathology: No test substance-related neoplasms or non-neoplastic findings occurred in males administered up to 2 mg/kg/day or females administered up to 3 mg/kg/day.
Neoplastic microscopic findings: The incidences and types of primary neoplasms that occurred in controls and animals administered MYK-461 were restricted to a few tissues/organs such as thymus, uterus, prostate, lung and blood vessels from multiple tissues (Summary Table 108; for all tissues examined, see Appendix B). For each tumor type, the sponsor performed statistical analysis if the incidence in at least one test-article treated group was increased by at least two occurrences over the control group for increasing incidence with dose. For tumors occurring in animals dying spontaneously or sacrificed in a moribund condition, the observation was classified by the sponsor as
1. Fatal: The tumor was a factor in the demise of the animal.
2. Non-fatal: The tumor was not a factor in the demise of the animal.
3. Uncertain
Fatal and non-fatal tumors were analyzed together, with a separate stratum for each. No tumors of uncertain context were noted. No observable or palpable (superficial as in mammary or skin) tumors were analyzed. Unadjusted P-values were reported for tumors. Where applicable, site or tumor combinations were statistically analyzed if the incidence in at least one test-article treated group was increased by at least two occurrences over the control group. The criteria for combination were based on Guidelines for combining neoplasms for evaluation of rodent carcinogenicity studies (McConnell et al., 1986). Based on statistical analyses for neoplastic lesions, the sponsor concludes that for both males and females, there were no statistically significant differences in tumor incidence (Table 109).

The FDA statistician (Dr. Feng Zhou) analysis showed no statistically significant positive dose response relationships among the MYK-461 treated groups and vehicle control group for male and female mice when data of the positive control group were excluded. There were also no statistically significant increases in incidence rate in test article treated group when compared with the vehicle control group.

The executive CAC concurred with the findings at the meeting held on July 20, 2021 (for meeting minutes, see Appendix E).

Table 108. Summary incidence of primary neoplastic findings (Groups 1 through 4)

	MYK-461							
Sex	Males				Females			
Dose Level MYK-461 (mg/kg/day)	0	0.5	1	2	0	0.5	1	3
Number of Animals	25	25	25	25	25	25	25	25
Vascular Neoplasms[a]								
B-Hemangioma	0	0	0	0	0	0	0	1
M-Hemangiosarcoma	3	1	4	2	5	7	3	4
Eye								
B-Melanoma, uveal, benign	0	0	0	0	0	0	1	0
Harderian Gland								
B-Adenoma	0	1	0	0	0	1	0	0
M-Adenocarcinoma	0	1	0	0	0	1	0	0
Lung								
B-Adenoma, bronchiolo-alveolar	4	2	2	4	0	0	1	1
M-Carcinoma, bronchiolo-alveolar	0	0	2	0	0	1	1	0
Prostate								
M-Sarcoma, NOS	0	0	1	0	0	0	0	0
Stomach, Nonglandular								
B-Papilloma, squamous cell	0	0	0	1	0	0	0	0
Thymus								
B-Thymoma, benign	0	0	0	0	3	1	1	1
M-Thymoma, malignant	0	1	0	0	0	1	0	0
Uterus								
B-Polyp, endometrial stromal	NA	NA	NA	NA	0	1	1	0

B = Benign; M = Malignant; NA = Not Applicable; NOS = Not otherwise specified.

a Present in multiple tissues, including the spleen, nonglandular stomach, rectum, testis, skin/subcutis, thymus, uterus, vagina, and sternum marrow.

Table 109. Results of statistical analyses for neoplastic lesions in males and females

Males

Group	Trend	Unadjusted Lifetime Incidence Rate 1	2	3	4
Dose Level (mg/kg/day)	(1,2,3,4)	0	0.5	1	2
Harderian Gland					
B-Adenoma/M-Adenocarcinoma					
No. Examined		25	25	25	24
Fatal Tumors		0	0	0	0
Incidental Tumors		0	2	0	0
Total Tumors		0	2	0	0
Peto P-value (v1)	0.8026	N/A	0.2347	N/A	N/A
Lung					
M-Carcinoma, bronchiolo-alveolar					
No. Examined		25	25	25	25
Fatal Tumors		0	0	0	0
Incidental Tumors		0	0	2	0
Total Tumors		0	0	2	0
Peto P-value (v1)	0.4158	N/A	N/A	0.4792	N/A
Spleen					
M-Hemangiosarcoma					
No. Examined		25	25	25	25
Fatal Tumors		0	0	1	0
Incidental Tumors		1	1	2	1
Total Tumors		1	1	3	1
Peto P-value (v1)	0.4911	N/A	N/A	0.2852	N/A

Females

Group	Trend	Unadjusted Lifetime Incidence Rate 1	2	3	4
Dose Level (mg/kg/day)	(1,2,3,4)	0	0.5	1	3
Harderian Gland					
B-Adenoma/M-Adenocarcinoma					
No. Examined		25	25	24	25
Fatal Tumors		0	0	0	0
Incidental Tumors		0	2	0	0
Total Tumors		0	2	0	0
Peto P-value (v1)	0.8197	N/A	0.2553	N/A	N/A
Lung					
B-Adenoma, bronchiolo-alveolar/					
M-Carcinoma, bronchiolo-alveolar					
No. Examined		25	25	25	25
Fatal Tumors		0	0	1	0
Incidental Tumors		0	1	1	1
Total Tumors		0	1	2	1
Peto P-value (v1)	0.3252	N/A	N/A	0.2553	N/A
Rectum/Skin/Subcutis/Spleen/					
Thymus/Uterus/Vagina					
B-Hemangioma/M-Hemangiosarcoma					
No. Examined		25	25	25	25
Fatal Tumors		1	1	0	1
Incidental Tumors		4	6	3	4
Total Tumors		5	7	3	5
Peto P-value (v1)	0.5955	N/A	0.4019	N/A	N/A

Microscopic findings in positive control animals indicated a clear carcinogenic response, consistent with the expected effect of MNU in the RasH2 transgenic mouse (Takaoka et al., 2003). The MNU-related primary neoplasms included malignant lymphoma in multiple locations, especially in the thymus, spleen, and/or other organs; squamous cell papilloma and/or carcinoma in the non-glandular stomach, oral mucosa, and/or skin/subcutis; and choriocarcinoma of the ovary correlating with ovary mass.

Non-neoplastic microscopic findings: Cardiac and slow skeletal muscle express the same myosin isoform, while cardiac and fast skeletal myosin are 80.7% identical. In in vitro pharmacodynamic studies (see Section 2.1), MYK-461 inhibited slow-skeletal bovine masseter myofibrils with equal potency (IC50, 0.43 µM) and fast skeletal muscle fibers with approximately 5-fold less potency (IC50, 2.14 µM). It suggests that this inhibition may have functional (skeletal) effects in vivo. In this 26-week study, multiple skeletal muscles (diaphragm, biceps femoris, gastrocnemius, quadriceps femoris, left soleus, and right soleus muscles) were examined microscopically from controls and animals administered MYK-461. A microscopic finding of degeneration/ regeneration, which was sometimes accompanied by infiltrates of mononuclear and/or mixed cells, occurred in the biceps femoris, gastrocnemius, and quadriceps femoris muscle of almost all animals from all groups, including controls; similar findings occurred at a lower incidence in the left and right soleus muscles of animals from all groups. The muscle degeneration/regeneration was characterized microscopically by variation in muscle fiber size, hyalinized muscle fibers, and/or centrally located nuclei that occasionally formed long chains. These microscopic findings were not attributed to MYK-461 as they were consistent with the spontaneously occurring skeletal muscle myopathy previously reported for this mouse strain (Tsuchiya et al., 2002). No degeneration/ regeneration of the muscle of the diaphragm was noted in any animal. The sponsor concludes that these expected muscle changes and all other non-neoplastic microscopic findings were considered spontaneous and/or incidental because they occurred at a low incidence, the incidence and/or severity of the findings were not dose related, and/or they were of the types expected for RasH2 mice (findings for all tissues examined are given in Appendix C) (Nambiar et al., 2012; Takaoka et al., 2003; Kanno et al., 2003; Morton et al., 2002; Paranjpe et al.; 2013a; Paranjpe et al., 2013b; Paranjpe et al., 2019).

Toxicokinetics: Following oral administration of MYK-461 at dose levels 0.5 to 2 mg/kg/day for males and 0.5 to 3 mg/kg/day for females, increases in maximum plasma concentration (Cmax) and exposure (AUC0-24) were both approximately

dose-proportional over the investigated Days 28 and 182. No accumulation of MYK-461 was noted with repeated dosing at any dose level or for either sex over the investigated period. Also, no sex differences in the TK parameters of MYK-461 were observed. Time to reach peak concentration was between 0.5 and 1 hr (Table 110).

Table 110. Toxicokinetic plasma parameters observed in male and female mice after daily oral gavage of MYK-461 for 26 weeks

Analyte	Day	Sex	Dose Level (mg/kg/day)	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24} (h*ng/mL)
MYK-461	28	M	0.5	711	0.5	6280
			1	1190	0.5	14500
			2	3140	0.5	34200
		F	0.5	612	0.5	7220
			1	1330	0.5	14800
			3	4520	1.0	49200
	182	M	0.5	768	1.0	6330
			1	1270	0.5	15800
			2	2230	1.0	30000
		F	0.5	626	0.5	6660
			1	1300	0.5	16400
			3	4280	0.5	50400

AUC0-t and AUC0-24 were equivalent

9 Reproductive and Developmental Toxicology

9.1 Fertility and early embryonic development to implantation of MYK-461 in rats

Conducting laboratory and location: (b) (4)

Testing facility study number: 20095223
Sponsor study number: NC-19-0028
Date of study initiation: June 14, 2016
Date of termination: Last day of in-life phase of the study was on August 5, 2016.
Drug lot/batch number: MYK-461, 140056, purity 99.93%
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

Oral administration of MYK-461 at doses up to 1.2 mg/kg/day in male and female rats once during the premating, mating and post-mating or gestation period (GD 7) was well tolerated. No deaths, clinical signs, gross lesions, or changes in mating, or fertility were attributed to administration of MYK-461 in either the male or female rats. The highest dose did not induce early embryonic development toxicity as evidenced by lack of effect on mean number of corpora lutea, implantations, and pre- and post-implantation losses. The NOAEL for fertility and early embryonic development to implantation of MYK-461 is 1.2 mg/kg/day, the highest dose tested.

Purpose

This study was designed to evaluate any potential adverse effects of MYK-461 on the estrous cycle, tubal transport, implantation, and development of preimplantation stages of the embryos of females and permit detection of functional effects (e.g., effects on libido or epididymal sperm maturation) that may not be detected by histological examinations of male reproductive organs.

Methods

Formulation

MYK-461 was suspended in aqueous 0.5% methylcellulose. It was prepared weekly, stored in a refrigerator and dispensed daily. The first and the last dose formulation samples were analyzed. The achieved concentrations met the acceptance criteria (i.e., ± 15% of nominal concentration).

Animals

Species/Strain: Rat (Crl:CD(SD); Sprague-Dawley)
#/Sex/Group: 22 (Table 111).
Age: Males were 71 days old, and the females were 66 days old at arrival.

Weight: Males: 325 to 384 gm, females: 216 to 275 gm

Husbandry: Animals were co-housed (2 per cage) by sex and by dose group until cohabitation. During the cohabitation period, a male and a female rat were pair housed for 11 days. Males that did not mate a female within the first 14 days of cohabitation were assigned alternate females from the same dose group and remained in cohabitation for a maximum of 7 additional days. After cohabitation, females were housed individually until the day of scheduled euthanasia. Food and water were given *ad libitum* throughout the study period.

Dosing

Suspensions of MYK-461 were administered orally by gavage, once daily, to groups of males and females at doses of 0.3, 0.6 or 1.2 mg/kg/day (Table 111). Males were dosed for 28 days before mating, throughout the mating period and continued a day before euthanasia. Thus, males were given 50 to 52 doses of test substance or vehicle (5 ml/kg body weight) prior to necropsy. Female rats received formulations daily for 15 days prior to mating with treatment continued until day 7 of presumed gestation. The doses were selected based of a previous 13-week toxicity study in the same rat strain. In this study, as a result of early adverse in-life changes, the high doses, 1 and 2 mg /kg/day were reduced to 0.6 and 1.2 mg/kg/day, respectively, from day 15 for the remainder of the study. There were no deaths in the study. Also, there were no test substance-related clinical signs in the surviving animals.

Table 111. Study design

Group No.	Test Material	Dose Level (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Rats (Assigned Numbers)	
					Males	Females
1	Control Article	0	0	5	22 (101-122)	22 (201-222)
2	MYK-461	0.3	0.06	5	22 (123-144)	22 (223-244)
3	MYK-461	0.6	0.12	5	22 (145-166)	22 (245-266)
4	MYK-461	1.2	0.24	5	22 (167-188)	22 (267-288)

Observations and Measurements

Clinical Signs: All animals were observed once daily during the dosing period and on the day of euthanasia for clinical signs. Post dose observations were recorded between 1 and 2 hours after dose administration.

Body Weights: Individual body weights were recorded twice weekly during the dosing period and once on the day of euthanasia for all animals. Additionally, females were weighed on gestation days 0, 3, 7, 10 and 13.

Food Consumption: For males, it was recorded weekly during the dosing period until the initiation of the cohabitation period. For females, food consumption was recorded weekly during the dosing period and on GDs 0, 3, 7, 10 and 13.

Estrous Cycle Evaluations: It was done by examining the vaginal cytology of samples obtained by vaginal lavage. Samples were collected for 14 consecutive days prior to dosing, for 14 consecutive days beginning with the day after the first dose administration, and then until spermatozoa were observed in a smear of the vaginal contents and/or a copulatory plug was observed in situ during the cohabitation period

Mating Performance: The cohabitation (pairing one male per one female) period consisted of 11 days and females with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed in situ were considered to be at GD 0 and assigned to individual housing. Males that did not mate a female within the first 14 days of cohabitation were assigned alternate females (same dose group) and remained in cohabitation for a maximum of 7 additional days.

Termination of Male Rats: Animals were euthanized during the study week 7 (after 50 to 52 doses) (Table 112). The potential toxicity of test substance on male reproductive system was assessed by evaluating sperm motility, concentration, and morphology.

Table 112. Terminal procedures for male rats

Group No.	No. of Male Rats	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology
			Sperm Analysis	Necropsy	Tissue Collection	Organ Weights		
1	22	Study Week 7[a]	X	X	X	X	-	-
2	22						-	-
3	22						-	-
4	22						-	-
Unscheduled Deaths			-	X	X	X	-	-

X = Procedure conducted; - = Not applicable.
[a] Euthanasia occurred after 50 to 52 doses of the test article.

Laparotomy: All surviving maternal rats were euthanized on GD 13 (Table 113) and thoracic, abdominal and cervical organs were macroscopically observed. Uterus and ovaries were excised and examined for number and distribution of corpora lutea, implantations, resorptions, live and dead fetuses. Fetuses were discarded without further examination.

Necropsy: For each animal, a gross necropsy of the thoracic, abdominal ad pelvic viscera was examined. Selected organs were collected (Table 114), weighted and organ to body weight ratio was determined. Representative samples of the tissues were collected (Table 114) from both males and females and preserved.

Table 113. Terminal procedures for female rats

Group No.	No. of Female Rats	Scheduled Euthanasia Day	Necropsy Procedures				Histology	Histopathology
			Ovarian/ Uterine Examination	Necropsy	Tissue Collection	Organ Weights		
1	22	DG 13	X	X	X	X	-	-
2	22						-	-
3	22						-	-
4	22						-	-
Unscheduled Deaths			-	-	-	-	-	-

X = Procedure conducted; - = Not applicable; DG = Day of presumed gestation.

Table 114. Tissue collection and preservation from all rats

Tissue	Weighed	Collected	Microscopic Evaluation	Comment
Cervix	-	X	-	Collected with uterus.
Epididymides	X	X	-	Individually weighed and examined. The remaining portion of the left epididymis (corpus and caput) and right epididymis were individually identified as to left or right and fixed in 10% NBF.
Epididymis, left cauda	X	X	-	Weighed for all males at scheduled euthanasia
Esophagus	-	X	-	Unscheduled death.
Gland, mammary	-	X	-	Collected with inguinal skin.
Gland, pituitary	-	X	-	-
Gland, prostate	X	X	-	Weighed for all males at scheduled euthanasia
Gland, seminal vesicle	X	X	-	Paired weight with fluid and examination. Weighed for all males at scheduled euthanasia.
Gross lesions/masses	-	X	-	-
Heart	-	X	-	Unscheduled death.
Kidney	-	X	-	Unscheduled death.
Liver	-	X	-	Unscheduled death.
Lung	-	X	-	Unscheduled death.
Ovaries	X	X	-	Individually weighed and examination.
Oviducts	-	X	-	Collected with uterus
Spleen	-	X	-	Unscheduled death.
Stomach	-	X	-	Unscheduled death.
Testes	X	X	-	Individually weighed and examination; individually identified as to left or right, fix in Bouin's solution for 48 to 96 hours, and retain in 10% NBF.
Trachea	-	X	-	Unscheduled death.
Uterus	-	X	-	Collected with cervix and oviduct.
Vagina	-	X	-	-

X = Procedure conducted; - = Not applicable.

Results

Mortality: Except for a male rat (#169) receiving 1.2 mg/kg/day, all other rats survived until scheduled necropsy. This rat was found dead on day 41 of study. There were no clinical observations prior to death and there were no gross findings at necropsy. The death was not considered related to the test substance administration.

Clinical Signs: A statistically significant increase in the number of males with mild dehydration was observed in the high dose group. However, the sign was transient and did not persist.

Body Weights: There was no effect in males. For females, statistically significant decreases ($P \leq 0.01$) in body weight gain were observed at 0.6 and 1.2 mg/kg/day on GDs 0 to 3 and 0 to 7 relative to control group value. However, the effect was not dose dependent (Fig. 47).

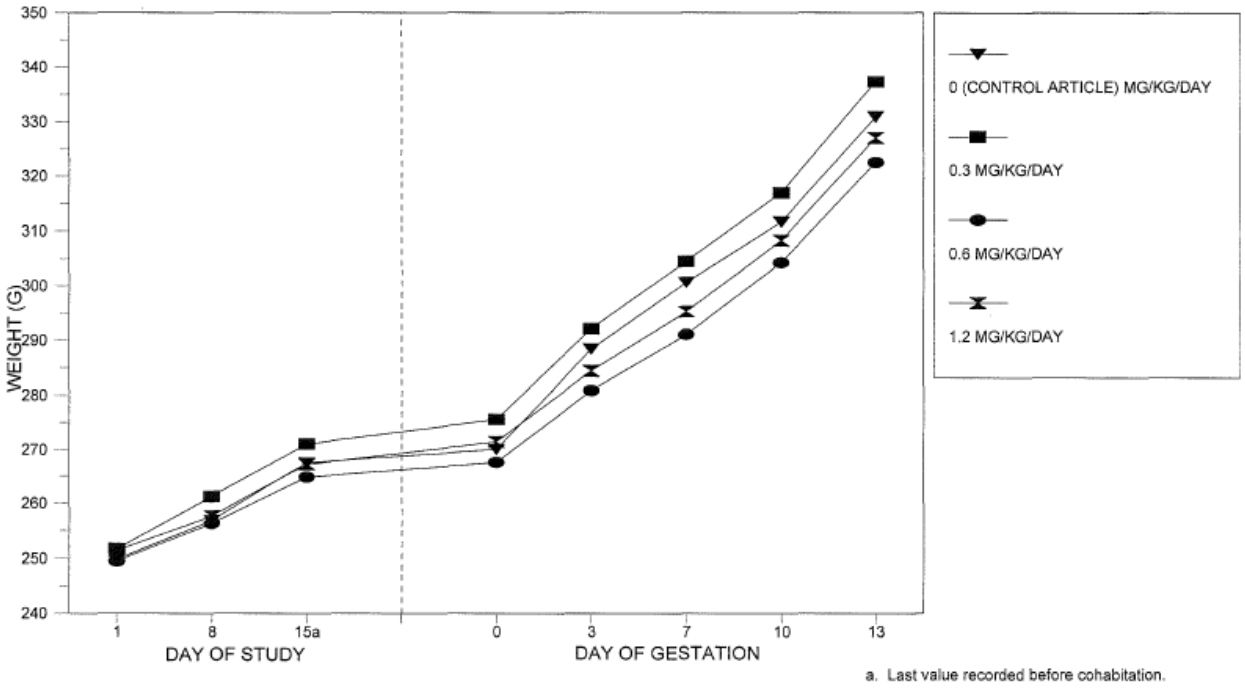


Figure 47. Body weights - female rats

Food Consumption: It was unaffected by the test substance in both male and female rats.

Reproductive Performance: In male rats, sperm motility, mean cauda epididymal sperm count and density and cauda epididymal sperm morphology parameters were generally comparable in all 4 dose groups. In female rats, the estrous cycle, mating behavior and pregnancy rate were unaffected by the test substance.

Pregnancy was confirmed in 22 (100%), 21 (95.4%), 19 (86.4%), and 20 (90.9%) treated females that were mated with treated males in the 0, 0.3, 0.6 and 1.2 mg/kg/day dose groups, respectively. MYK461 had no effect on any ovarian or uterine parameters at any dose level. The mean number of corpora lutea, implantations, percent pre- and post-implantation losses were similar across all groups. No dam had a litter consisting of only nonviable embryos. None of the placenta examined had any detectable abnormalities (Table 115).

Necropsy: There were no MYK-461-related macroscopic observations detected in male or female rats at necropsy examination. Additionally, there was no effect on organ weights in all rats.

Table 115. Summary of ovarian and uterine contents: female rats

GROUP		1	2	3	4
TEST MATERIAL		CONTROL ARTICLE	MYK-461	MYK-461	MYK-461
DOSE LEVEL (MG/KG/DAY)a		0	0.3	0.6	1.2
RATS TESTED	N	22	22	22	22
PREGNANT	N(%)	22(100.0)	21(95.4)	19(86.4)	20(90.9)
RATS PREGNANT AND CAESAREAN-SECTIONED ON DAY 13 OF GESTATION	N	22	21	19	20
CORPORA LUTEA	MEAN±S.D.	18.0 ± 2.9	17.3 ± 2.3	16.5 ± 2.2	16.5 ± 2.3
IMPLANTATIONS	MEAN±S.D.	16.0 ± 1.8	16.3 ± 1.7	15.1 ± 3.4	15.2 ± 2.0
% PREIMPLANTATION LOSS	MEAN±S.D.	9.9 ± 9.2	5.2 ± 7.5	8.9 ± 17.9	7.5 ± 6.4
VIABLE EMBRYOS	N	339	330	274	290
	MEAN±S.D.	15.4 ± 1.9	15.7 ± 1.7	14.4 ± 3.2	14.5 ± 2.4
NONVIABLE EMBRYOS	N	13	12	13	14
	MEAN±S.D.	0.6 ± 0.7	0.6 ± 0.7	0.7 ± 0.7	0.7 ± 1.3
% POSTIMPLANTATION LOSS	MEAN±S.D.	3.7 ± 4.6	3.4 ± 4.5	4.3 ± 4.4	4.7 ± 8.7
DAMS WITH ANY NONVIABLE EMBRYOS	N(%)	10(45.4)	9(42.8)	10(52.6)	8(40.0)
DAMS WITH ALL NONVIABLE EMBRYOS	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DAMS WITH VIABLE EMBRYOS	N(%)	22(100.0)	21(100.0)	19(100.0)	20(100.0)
PLACENTAE APPEARED NORMAL	N(%)	24(100.0)	23(100.0)	19(100.0)	19(100.0)

% PREIMPLANTATION LOSS = [(NUMBER OF CORPORA LUTEA - NUMBER OF IMPLANTATIONS)/NUMBER OF CORPORA LUTEA] X 100
% POSTIMPLANTATION LOSS = [(NUMBER OF IMPLANTATIONS - NUMBER OF LIVE FETUSES)/NUMBER OF IMPLANTATIONS] X 100
a. Dose administration occurred on Day 1 of study through Day 7 of gestation.

9.2 Embryo-fetal toxicity study of MYK-461 in rats

Conducting laboratory and location: (b) (4)

Testing facility study number: TER0690
Sponsor study number: NC-16-0004
Date of study initiation: November 17, 2015
Date of termination: Last day of cesarean section: December 17, 2015
Drug lot/batch number: MYK-461, #15B0013 ((b) (4) lot 150030), purity 99.87%
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

Oral administration of MYK-461 from GD 6 through 12 at doses up to 1.5 mg/kg/day in rats did not cause maternal death or decrease in body weight gain and food consumption. However, the high dose resulted in developmental toxicity as evidenced by increased post-implantation loss, altered fetal growth (such as lower fetal body weight and reduced fetal ossification of bones (cervical and thoracic vertebrae and paws (phalanxes and metatarsals)), visceral (heart) malformations (consisted of total situs inversus (thoracic and abdominal) in one fetus and absence of the right atrioventricular valve associated with a ventricular septum defect in the second fetus) and skeletal malformations (fused sternebrae and absence of lumbar vertebrae). MYK-461 is teratogenic with a NOAEL of 0.75 mg/kg/day for developmental toxicity. The NOAEL for maternal toxicity was 1.5 mg/kg/day.

Purpose

This study was designed to determine the potential embryo-fetal and maternal toxicity of MYK-461 when administered once daily, orally, to pregnant rats from Gestation Days (GD) 6 through 17. The study evaluated the effects of MYK-461 during the period of organogenesis, from implantation to closure of the hard palate. Blood samples for toxicokinetic evaluation of MYK-461 and its enantiomer MYK-460 in pregnant rats were also determined.

Methods

Formulation

MYK-461 was suspended in aqueous 0.5% methylcellulose. It was stable for 2 weeks at room temperature or refrigerator conditions. Frequency of preparation is not given. Formulation was analyzed at the beginning of the first week of treatment. The achieved concentrations met the acceptance criteria (i.e., 80 to 120% of nominal concentration).

Animals

Species/Strain: Rat (Crl:CD(SD); Sprague-Dawley)
#/Sex/Group: 24 (for main study) plus 3 (for TK) pregnant females/group
Age: 9-11 weeks old at the start of dosing
Weight: 234.32 to 308.27 gm
Husbandry: Pregnant females were housed individually in cages. Food and water were given *ad libitum* throughout the study period.

Dosing

Suspensions of MYK-461 were administered orally by gavage, once daily, to groups of presumed pregnant females at doses of 0.3, 0.75 or 1.5 mg/kg/day (Table 116). The control animals received the vehicle (5 ml/kg body weight). Main study animals were treated from GD 6 to GD 17, while TK animals were treated from GD 6 to GD 12. The doses were selected on the basis of a previous exploratory embryo-fetal study (#TEP0362) in the same rat strain in which MYK-461 was administered to pregnant rats up to 2 mg/kg/day for 12 days. Test substance produced decreases in body weight gain, increased post-implantation loss and lower mean fetal body weight at ≥1.5 mg/kg/day.

Table 116. Study design

Group	Dosage (mg/kg/day)	Number of mated females(Main Study)	Animal numbers	Number of mated females [Toxicokinetics (TK)]	TK Animal numbers
1 [a]	0	24	1-24	3	97-99
2	0.3	24	25-48	6	100-105
3	0.75	24	49-72	6	106-111
4	1.5	24	73-96	6	112-117

[a] An aqueous solution of 0.5% (w/w) methylcellulose (400 cps)

Observations and Measurements

Clinical Signs: All main study animals were observed once daily on GD 1 to 5 and GD 18 to 21 and once before and once after the dose (1 to 4 h postdose) for clinical signs. They were observed twice daily for death and abortion.
Body Weights: Individual body weights were recorded for all animals on gestation days 1, 6, 7, 9, 12, 15, 18 and 21.
Food Consumption: Recorded for main study animals on gestation days 1, 6, 9, 12, 15, 18 and 21.
Laparotomy: All surviving maternal rats in the main study were euthanized on GD 21 and thoracic, abdominal and cervical organs were macroscopically observed. Uterus, ovaries and placenta of the fetus were excised from the animal and weighed. The gravid uterus was excised and weighed. The numbers of corpora lutea, implantations, resorptions, live and dead fetuses were recorded. Each

viable fetus was sexed, weighed and examined externally for gross abnormalities. Skeletal examinations were conducted on approximately 50% of the fetuses of each litter. The remaining fetuses were examined for visceral alterations. The head and the heart were removed and placed in solution for examination by section. The carcasses were later discarded. TK animals were euthanized after the last sampling time point on GD 13 and discarded without further examination after determination of pregnancy status.
Toxicokinetics: Blood samples for test substance determination were collected at 0.5, 1, 2, 4, 8 and 24 h (3 animals per time point) after dosing on the study day 12 from the tail vein of TK animals. Blood samples from control animals were collected at 2 and 24 h after dosing. Animals were not fasted.

Results

Mortality: There were no deaths except one in the control group on GD 6.
Clinical Signs: No signs of toxicity were observed.
Body Weights: A minimal decrease ($P < 0.05$) in body weight gain was noted between GDs 6 and 9, and GDs 12 and 15 for the high dose group. This effect was considered to be within the expected biological variation.
Food Consumption: No effect
Toxicokinetics: The systemic exposure (Cmax and AUC) increased more than dose proportionately on all days of measurement. Peak concentration was at 0.5 h post dosing (Table 117). Interconversion of MYK-461 to its enantiomer, MYK-460, after repeat-dose administration was not observed.

Table 117. MYK-461 toxicokinetic parameters in plasma on GD12

Dose (mg/kg/day)	t_{max} (h)	C_{max} (ng/mL)	AUC_{0-24} (ng.h/mL)
0.30	0.50	116	1520
0.75	0.50	356	5690
1.50	0.50	1080	16500

Reproductive Performance: The mean terminal body weight and uterus weight of all treated groups were comparable to that of the control group. There were no treatment-related macroscopic observations. The pregnancy rate, mean number of corpora lutea, implantations and preimplantation loss were similar across the groups. However, post-implantation loss characterized by an increase in early and late resorptions (1.52 resorptions vs 0.87 in control) was statistically significantly higher at 1.5 mg/kg/day than in control. A slightly large litter size was

noted at 0.3 and 0.75 mg/kg/day (Summary Table 118; for details, see Table 119). A dose-dependent statistically significant trend in decrease in mean fetal body weight was noted in all dose groups (-3%, -4% and -11% in low, mid and high dose groups, respectively) relative to control (Table 119).

Table 118. Summary of reproductive performance and litter data (means)

Group No.	1	2	3	4	Historical control data [a]
Dose level (mg/kg/day)	**0**	**0.3**	**0.75**	**1.5**	**N or Mean [Min-Max]**
Number of surviving pregnant females	23	24	24	23	456
Corpora Lutea (mean number)	15.8	16.3	16.3	16.0	15.0 [13.5 – 16.5]
Implantation Sites (mean number)	13.5	15.0	15.0	14.4	13.8 [11.7 – 15.4]
Post-implantation Loss (total) (mean number)	0.87	0.63	0.96	1.52	1.3 [0.5 – 2.6]
Live fetuses (mean number)	12.7	14.3	14.0	12.9	12.6 [10.5 – 14.3]
Mean Live Fetal body weight (g)	5.80	5.64	5.59	5.16 (-11%)	5.41 [4.88 – 5.67]

Statistically significant relative to control is indicated in underlined numbers.

Fetal examination: There were no treatment-related external malformations or variations in fetuses treated with MYK-461. However, visceral and skeletal anomalies were observed in the high dose group relative to the control group. A higher number of fetuses were presented with at least one malformation at 1.5 mg/kg/day (Table 120). Visceral examination showed 2 malformed fetuses (one with total situs inversus (thoracic and abdominal) and the 2nd presenting with heart malformation (absence of the right atrioventricular valve associated with a ventricular septum defect) from two different litters at 1.5 mg/kg/day. Visceral variations (minor anomalies) were also noted in the subclavian artery (malpositioned branch), thymus (misshapen), dilated renal pelvis(es), and/or ureter (convoluted and/or dilated), but were considered unrelated to test substance administration by the sponsor since they occurred in control animals, at incidences comparable to those of the test facility historical control data.

Table 119. Summary of female cesarean section, early embryonic and litter data

Dose mg/kg/d		Corpora Lutea	-Preimplantation- -Loss- Absolute (c)	-Preimplantation- -Loss- %Corpora Lutea	Implant Sites (c)	Postimplantation Loss: Absolute: ---Resorptions--- Early	Resorptions Late	Dead Fetuses	Total (i)	%Implantations	Live Fetuses: Absolute (i)	%Implantations	Live Fetuses: Total (i)	Percent Males	Mean Live Fetal Wt (g): Male	Female	Total
Group 1	MEAN	15.8	2.3	14.5	13.5	0.8	0.0	0.0	0.87	6.2	12.7	93.8	12.7	48.6	6.00	5.59	5.80
0	STD	1.9	2.9	18.8	3.2	0.9	0.2	0.0	0.87	6.1	3.1	6.1	3.1	15.8	0.27	0.27	0.24
	MEDIAN	16.0	1.0	6.7	14.0	1.0	0.0	0.0	1.00	6.7	14.0	93.3	14.0	50.0	6.02	5.55	5.75
	MIN	12.0	0.0	0.0	5.0	0.0	0.0	0.0	0.00	0.0	5.0	80.0	5.0	15.4	5.49	5.10	5.32
	MAX	20.0	11.0	61.1	19.0	3.0	1.0	0.0	3.00	20.0	17.0	100.0	17.0	85.7	6.49	6.16	6.29
	N	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
Group 2	MEAN	16.3	1.3	7.4	15.0	0.6	0.0	0.0	0.63	4.1	14.3	95.9	14.3	48.4	5.83 *	5.47 NS	5.64 *
0.3	STD	2.4	1.3	7.3	1.9	0.7	0.2	0.0	0.82	5.5	2.0	5.5	2.0	13.3	0.36	0.28	0.31
	MEDIAN	17.0	1.0	5.9	15.0	0.0	0.0	0.0	0.00	0.0	14.0	100.0	14.0	48.1	5.80	5.41	5.64
	MIN	10.0	0.0	0.0	10.0	0.0	0.0	0.0	0.00	0.0	10.0	80.0	10.0	23.1	5.08	4.94	5.02
	MAX	20.0	4.0	21.1	19.0	2.0	1.0	0.0	3.00	20.0	19.0	100.0	19.0	81.8	6.62	5.91	6.19
	% control	+3	-44	-49	+11	-29	-4		-28	-34	+13	+2	+13	0	-3	-2	-3
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Group 3	MEAN	16.3	1.3	8.0	15.0	1.0	0.0	0.0	0.96 NS	7.6	14.0 NS	92.4	14.0 NS	47.5	5.72 *	5.46 *	5.59 *
0.75	STD	3.2	1.3	8.3	3.0	1.2	0.0	0.0	1.20	11.4	3.6	11.4	3.6	17.1	0.32	0.28	0.29
	MEDIAN	17.0	1.0	5.9	16.0	1.0	0.0	0.0	1.00	6.3	15.0	93.8	15.0	45.4	5.85	5.48	5.61
	MIN	8.0	0.0	0.0	6.0	0.0	0.0	0.0	0.00	0.0	4.0	50.0	4.0	0.0	4.99	4.90	4.99
	MAX	21.0	5.0	25.0	19.0	4.0	0.0	0.0	4.00	50.0	19.0	100.0	19.0	75.0	6.20	5.89	6.05
	% control	+3	-44	-45	+11	+16	-100		+10	+23	+11	-2	+11	-2	-5	-2	-4
	N	24	24	24	24	24	24	24	24	24	24	24	24	24	23	24	24
Group 4	MEAN	16.0 NS	1.7 NS	10.5	14.4 NS	1.3	0.2	0.0	1.52 *	10.2	12.9 *	89.8	12.9 *	51.2 NS	5.28 *	5.02 *	5.16 *
1.5	STD	2.0	2.2	15.0	2.9	1.8	0.5	0.0	1.86	12.8	3.2	12.8	3.2	14.7	0.33	0.34	0.32
	MEDIAN	16.0	1.0	6.3	15.0	1.0	0.0	0.0	1.00	6.3	13.0	93.8	13.0	50.0	5.19	5.07	5.09
	MIN	13.0	0.0	0.0	5.0	0.0	0.0	0.0	0.00	0.0	5.0	57.1	5.0	20.0	4.88	4.37	4.78
	MAX	22.0	9.0	64.3	19.0	6.0	2.0	0.0	6.00	42.9	18.0	100.0	18.0	80.0	6.11	5.80	5.96
	% control	+1	-28	-28	+6	+63	+300		+75	+64	+2	-4	+2	+5	-12	-10	-11
	N	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23

* = Significant trend ($p <= 0.05$) through indicated dose level
NS = No significant trend through indicated dose level
NT = No test performed
i = Analysis stratified by number of implantation sites
c = Analysis stratified by number of corpora lutea

Table 120. Number of malformed fetuses by exam type

Group	1	2	3	4
Dose Level (mg/kg/day)	**0**	**0.3**	**0.75**	**1.5**
Number of Live Fetuses (Litter)	**291 (23)**	**344 (24)**	**336 (24)**	**296 (23)**
Number of malformed live fetuses (litters) [a]	4 (2)	4 (3)	-	27 (13)
EXTERNAL	2 (1)	2 (2)	-	1 (1)
VISCERAL	-	-	-	2 (2)
SKELETAL	2 (1)	2 (2)	-	24 (12)

Incidence is the number of affected fetuses (affected litters)

[a] One live fetus may have more than one anomaly

Skeletal examination: Test substance-related skeletal malformations were noted with higher frequency at 1.5 mg/kg/day than in any other groups including control. The sponsor notes that findings on lumbar vertebra (missing vertebra noted in 2 fetuses from one litter and lumbar arch from 3 fetuses), and sternebra (fused sternebrae noted in 19 fetuses from 10 litters) were recorded at higher incidence in the high dose group than those of the test facility historical control data. No malformations were observed in other groups with the exception of a rib malformation (short 13th rib) noted in control, 0.3 and 1.5 mg/kg/day groups with low incidence (2/151, 2/179 and 5/156 fetuses, respectively), in the range of historical control data (up to 3.8%) (Table 121). Additionally, skeletal minor anomalies (variations) such as supernumerary ribs and sternebra variation were found in a few fetuses in all groups, including controls, with no clear dose-related incidence and were within the range of historical control data. Increased incidence of incomplete ossification (e.g., lumbar arch, cervical vertebra, and paws (phalanxes and metatarsals)) relative to control was also noted in the high dose group (Table 121). Although the incidence of ossification for some bones in the high dose group is close to historical control range for this strain but exceeded the concurrent control. According to the sponsor this is related to the lower fetal body weight at the high dose.

In conclusion, MYK-461 at 1.5 mg/kg/day was maternally well tolerated but resulted in development toxicity evidenced by increased post-implantation loss, altered fetal growth (i.e., lower mean fetal body weight, and slightly reduced fetal skeletal ossification), visceral malformations (heart malformation in 2 fetuses, including one total situs inversus) and increased incidences of skeletal malformations (mainly fused sternebrae) when compared to concurrent and

historical controls. MYK-461 is teratogenic with a NOAEL of 0.75 mg/kg/day for developmental toxicity. The NOAEL for maternal toxicity was 1.5 mg/kg/day.

Table 121. Summary of skeletal morphological findings in live fetuses

REPORT: SKELETAL EXAM TYPE: SKELETAL	CLASSIFICATION	GROUP 1 0 mg/kg/d NO	 %	GROUP 2 0.3 mg/kg/d NO	 %	GROUP 3 0.75 mg/kg/d NO	 %	GROUP 4 1.5 mg/kg/d NO	 %	MINIMUM-MAXIMUM RANGE OF HISTORICAL CONTROLS (PER STUDY) %
NUMBER OF FETUSES EXAMINED		151		179		174		156		
NUMBER OF LITTERS EXAMINED		23		24		24		23		
CERVICAL VERTEBRAE										
CERVICAL VERTEBRA INCOMPLETELY OSSIFIED	OSS	9	6.0	17	9.5	10	5.7	26	16.7	0.0 - 10.9
		7	30.4	8	33.3	8	33.3	15	65.2	0.0 - 34.8
THORACIC VERTEBRAE										
THORACIC VERTEBRA INCOMPLETELY OSSIFIED	OSS	1	0.7	2	1.1	1	0.6	3	1.9	0.0 - 2.7
		1	4.3	2	8.3	1	4.2	3	13.0	0.0 - 13.6
LUMBAR VERTEBRAE										
LUMBAR VERTEBRA MALFORMATION	MAL	0	0.0	0	0.0	0	0.0	2	1.3	0.0 - 0.4
		0	0.0	0	0.0	0	0.0	1	4.3	0.0 - 2.5
LUMBAR ARCH ISOLATED OSSIFICATION SITE	OSS	0	0.0	2	1.1	0	0.0	3	1.9	0.0 - 0.0
		0	0.0	1	4.2	0	0.0	2	8.7	0.0 - 0.0
RIBS										
RIB MALFORMATION	MAL	2	1.3	2	1.1	0	0.0	5	3.2	0.0 - 3.8
		1	4.3	2	8.3	0	0.0	2	8.7	0.0 - 22.7
SUPERNUMERARY RIB	MIN	3	2.0	13	7.3	10	5.7	13	8.3	0.0 - 12.2
		3	13.0	9	37.5	8	33.3	7	30.4	0.0 - 43.5
STERNEBRAE										
STERNEBRA VARIATION	MIN	1	0.7	2	1.1	0	0.0	1	0.6	0.0 - 3.3
		1	4.3	2	8.3	0	0.0	1	4.3	0.0 - 16.7
STERNEBRA MALFORMATION	MAL	0	0.0	0	0.0	0	0.0	19	12.2	0.0 - 0.8
		0	0.0	0	0.0	0	0.0	10	43.5	0.0 - 4.5

UPPER LINE: NUMBER OF AFFECTED FETUSES LOWER LINE: NUMBER OF AFFECTED LITTERS
MAL: MALFORMATION MIN: MINOR ANOMALY OSS: OSSIFICATION

9.3 Embryo-fetal toxicity study of MYK-461 in rabbits

Conducting laboratory and location: (b) (4)

Testing facility study number: TER0689
Sponsor study number: NC-16-0051
Date of study initiation: January 10, 2016
Date of termination: Last day of cesarean section: February 18, 2016
Drug lot/batch number: MYK-461, #15B0013 ((b) (4) lot 150030), purity 99.87%
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

Oral administration of MYK-461 from GD 6 through 19 at the high dose of 2.0 mg/kg/day caused 2 maternal deaths accompanied by marked body weight loss and little food intake. Bilateral ventricular dilation without any associated microscopic evidence suggests heart failure as the probable cause of death. Surviving animals at ≥1.2 mg/kg/day revealed a mild body weight loss or marked decrease in body weight gain along with a decreased food intake during the dosing period. While there was no effect on pregnancy and reproductive parameters, developmental toxicity as evidenced by test substance-related external (cleft palate), visceral (great vessels) and skeletal (fused sternebrae) malformations and incomplete ossification of a few bones were noted at ≥1.2 mg/kg/day. MYK-461 was quantified in embryonic and extra-embryonic (fetal envelopes, placenta, amniotic fluid) tissues in pregnant animals on GD 12, the earliest day of measurement. Individual embryonic tissue to plasma concentration ratios ranged between 0.09 and 0.15 in proportion with the dose administered. MYK-461 is teratogenic with the NOAEL of 0.6 mg/kg/day for maternal and developmental toxicity.

Purpose

This study was designed to determine the potential embryo-fetal and maternal toxicity of MYK-461 when administered once daily, orally, to pregnant rabbits from gestation Days (GD) 6 through 19. The study evaluated the effects of MYK-461 during the period of organogenesis, from implantation to closure of the hard palate. Blood samples for toxicokinetic evaluation of MYK-461 and its enantiomer MYK-460 in pregnant rabbits were also determined.

Methods

Formulation

MYK-461 was suspended in aqueous 0.5% methylcellulose. It was stable for 2 weeks at room temperature or refrigerator conditions. Frequency of preparation is not given. Formulation was analyzed at the beginning of the first week of

treatment, at mid-study and on the first day of blood sampling for toxicokinetic determination at the beginning of the first week of treatment. The achieved concentrations met the acceptance criteria (i.e., 85 to 115% of nominal concentration).

Animals

Species/Strain: Rabbits / Lago:INR(NZW) New Zealand White

#/Sex/Group: 22 (for main study) plus 3 (for TK) pregnant females/group

Age: 18-21 weeks old at the start of dosing

Weight: 234.32 to 308.27 gm

Husbandry: Mated females were housed individually in cages. Measured quantity of food and unlimited supply of water were provided throughout the study period.

Dosing

Suspensions of MYK-461 were administered orally by gavage, once daily, to groups of presumed pregnant females at doses of 0.6, 1.2 or 2.0 mg/kg/day (Table 122). The control animals received the vehicle (5 ml/kg body weight). Main study animals were treated from GD 6 to GD 19, while TK animals were treated from GD 6 to GD 12. The doses were selected on the basis of a previous exploratory embryo-fetal study (#TEP0361) in the same rat strain in which MYK-461 was administered to pregnant rats up to 2 mg/kg/day for 14 days. Test substance produced decreases in body weight gain at ≥1.5 mg/kg/day and increased post-implantation loss at 2 mg/kg/day.

Table 122. Study design

Group	Dosage (mg/kg/day)	Number of mated females (Main study)	Animal numbers	Number of mated females (TK)	TK Animal numbers
1 [a]	0	22	1-22	3	89-91
2	0.6	22	23-44	3	92-94
3	1.2	22	45-66	3	95-97
4	2	22	67-88	3	98-100

[a] An aqueous solution of 0.5% (w/w) methylcellulose (400 cps)
TK: Toxicokinetics

Observations and Measurements

Clinical Signs: All main study animals were observed once daily on GD 1 to 5 and GD 20 to 29 and once before and once after the dose (1 to 4 h postdose) during the dosing period for clinical signs. They were observed twice daily for death and abortion.

Body Weights: Individual body weights were recorded for all animals on gestation days 1, 6, 7, 9, 12, 15, 18, 20, 25 and 29.

Food Consumption: Recorded for main study animals on a daily basis from gestation day 1. For TK animals, it was measured on GD 1 and GD 6.
Laparotomy: All surviving maternal rabbits in the main study were euthanized on GD 29 and thoracic, abdominal and cervical organs were macroscopically observed. Similar procedure was followed for aborted animals and unscheduled deaths. Selected tissues with macroscopic observations (females #01, 10, 45, 46, 51, 65, 71, and 83) and heart (#10, 65, 71, and 83) were collected, preserved and examined microscopically. Uterus, ovaries and placenta of the fetus were excised from the animal and weighed. The pregnancy status, numbers of corpora lutea, implantations, resorptions (early and late), live and dead fetuses were recorded. Each viable fetus was sexed, weighed and examined externally for gross abnormalities. All fetuses were examined for visceral alterations. The head and the heart were removed and placed in solution for examination by section. Skeletal examinations were conducted on all fetuses. The carcasses were later discarded.
Toxicokinetics: Blood samples for test substance determination were collected at 0.5, 1, 2, 4, 8 and 24 h (3 animals per time point) after dosing on GD 12 from the central ear artery of TK animals. Blood samples from control animals were collected at 2 and 24 h after dosing. Animals were not fasted. Animals were euthanized after the last blood sample collection on GD 13. With cesarean section, pregnancy status was recorded, and embryos and extra-embryonic tissues were collected and frozen. Animals were discarded without further examination.

Results

Mortality: Two pregnant females (#71 and #83) at 2 mg/kg/day were found dead on GDs 16 and 18, respectively (Table 123). Both animals showed marked body weight loss (-10 to -12% relative to GD 6) associated with little food consumption for approximately a week prior to their death. Although macroscopic evidence revealed mild bilateral ventricular dilation there was no microscopic evidence in the heart, lungs or liver to suggest a heart failure. Additionally, three pregnant animals (1 each from control (#10), low (#37) and mid (#45) dose groups) were euthanized on GDs 25, 21 and 24, respectively, as a result of abortion. The control animal demonstrated body weight loss (-14% between GD 6 and GD 25) and both the control and the low dose group animals consumed a little food 2 days prior to abortion. Both macroscopic and microscopic findings presented an evidence of faulty gavage administration of the drug. Animal #45 did not present any evidence for death and the sponsor suggests incidental death (Table 123).
Clinical Signs: No test substance clinical signs of toxicity were observed in surviving rabbits.

Table 123. Mortality- findings noted in pre-terminally euthanized animals

Group Number	Female Number	Death Status	Day of death	Main in-life changes prior to death	Macroscopic and/or microscopic observations	
1	10	AE [a]	25	Body weight loss (-14% between GD6 and GD25) Very low food intake (≤30 g/day) during GD12-20	Heart	No macroscopic findings. Infiltrate, mononuclear cell, minimal, multifocal/ epicardial fatty tissue and myocardium.
					Lung	Infiltrate, mixed cells, multifocal, moderate/ Pleura. Consisting of macrophages, multinucleated giant cells, plasma cells, lymphocytes and heterophils, with extension to the lung parenchyma *. Necrosis, pleura, diffuse, marked / with fibrinous material and cellular debris **. Neovascularization, diffuse, marked/ pleura * Alveolar macrophages, multifocal, mild. * Correlated with: "LUNG; Focus/area; dark; bilateral; multifocal; moderate" and "LUNG; Discoloration; red; bilateral; diffuse; marked/severe". ** Correlated with: "LUNG; Abnormal content; white; right; moderate".
2	37	AE	21	-		No macroscopic findings. No microscopic examination.
3	45	AE	24	Very low food intake (≤30 g/day) during GD22-23	Lung	Alveolar macrophages, multifocal, mild Infiltrate, mixed cells, multifocal, minimal/interstitial *. Hyperplasia, bronchial epithelium, multifocal, mild *. Hyperplasia, bronchus-associated lymphoid tissue, diffuse, mild *. * Correlated with: "LUNG; Focus/area; red; bilateral; multifocal; moderate"
					Stomach	No abnormality detected at microscopic examination for "STOMACH; Focus/area; red; focal; minimal/mild" Focal and minimal hemorrhage was present at the junction with duodenum (on the duodenal side).
4	71	FD [b]	16	Body weight loss (-12% between GD6 and GD15) Very low food intake (≤30 g/day) during GD8-15	Heart	No macroscopic findings. Dilatation, ventricle, mild / both ventricles.
					Liver	Tissue autolytic but readable. Vacuolation, hepatocytes, diffuse, moderate *. * Correlated with: "LIVER; abnormal texture; granular; moderate".
					Lung	Alveolar macrophages, multifocal, minimal *. * Correlated with "LUNG; Discoloration; light/pale; right; focal; minimal/mild".
					Stomach	Tissue autolytic but readable. No abnormality detected at microscopic examination for "STOMACH; abnormal texture; red; multifocal; minimal/mild".
4	83	FD	18	Body weight loss (-10% between GD6 and GD18) Very low food intake (≤30 g/day) during GD12-17	Heart	No macroscopic findings. Dilatation, ventricle, mild / both ventricles. Hemorrhage, right atrio-ventricular valve, focal, mild.
					Liver	Tissue autolytic but readable. Vacuolation, hepatocytes, diffuse, moderate *. * Correlated with: "LIVER; discoloration; mottled; moderate" and "LIVER; Abnormal texture; friable/brittle; moderate".
					Lung	Small lungs (bilateral) were recorded at necropsy. Alveolar macrophages, multifocal, mild. Infiltrate, mixed cells, multifocal, minimal.

Body Weights: Dams receiving ≥1.2 mg/kg/day did not gain body weight but rather lost body weight (ranging from -0.8% to -9.6%) in a dose-dependent manner during the dosing period (Table 124). At 1.2 mg/kg/day, the overall body weight gain during the dosing period was less (-74%) than that of control as a consequence of moderate lower food intake than the control. Animals receiving 2 mg/kg/day steadily lost body weight for the entire dosing period (Fig. 47) relative to control. The changes from control were between -101 and 206%. Animals in both dose groups did not fully regain the body weight by GD 29 (Fig. 47).

Table 124. Summary of pregnant rabbit body weight changes through gestation

Dose mg/kg/d		Day 6-9	Day 6-12	Day 6-15	Day 6-18	Day 6-20	Day 6-25	Day 6-29
Group 1 0	MEAN	0.025	0.045	0.094	0.094	0.115	0.192	0.193
	STD	0.035	0.044	0.080	0.114	0.141	0.156	0.170
	N	20	20	20	20	20	20	20
Group 2 0.6	MEAN	0.022	0.038	0.057	0.083	0.098	0.152	0.176
	STD	0.031	0.052	0.093	0.109	0.105	0.124	0.116
	% control	-14	-16	-39	-12	-15	-21	-9
	N	19	19	19	19	19	19	19
Group 3 1.2	MEAN	0.002	-0.005	0.024	0.023	0.030	0.091	0.102
	STD	0.069	0.108	0.146	0.160	0.170	0.188	0.205
	% control	-90	-111	-75	-76	-74	-52	-47
	N	21	21	21	21	21	21	21
Group 4 2	MEAN	-0.012	-0.048	-0.062	-0.066	-0.064	-0.002	0.029
	STD	0.037	0.087	0.141	0.178	0.174	0.232	0.180
	% control	-146	-206	-166	-170	-155	-101	-85
	N	20	20	20	20	20	20	20

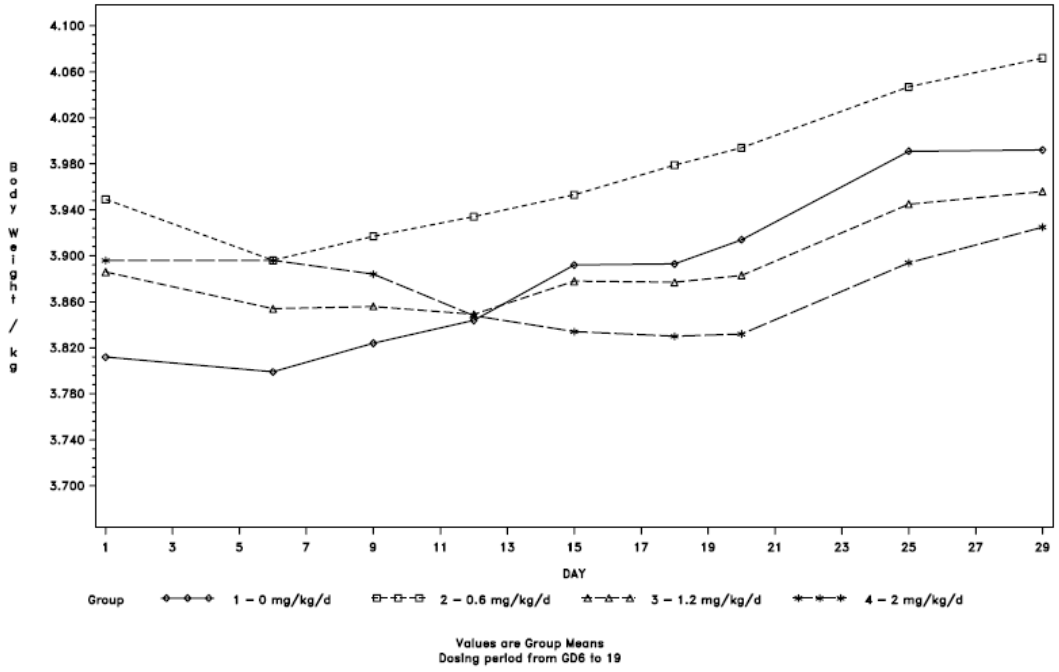


Figure 48. Pregnant rabbit body weight during gestation days 1 through 29

Food consumption: Dose-related decreases ($P < 0.05$) in food consumption relative to control were noted at ≥1.2 mg/kg/day for the entire dosing period (Fig. 49). Food intake was comparable to control after the dosing period.

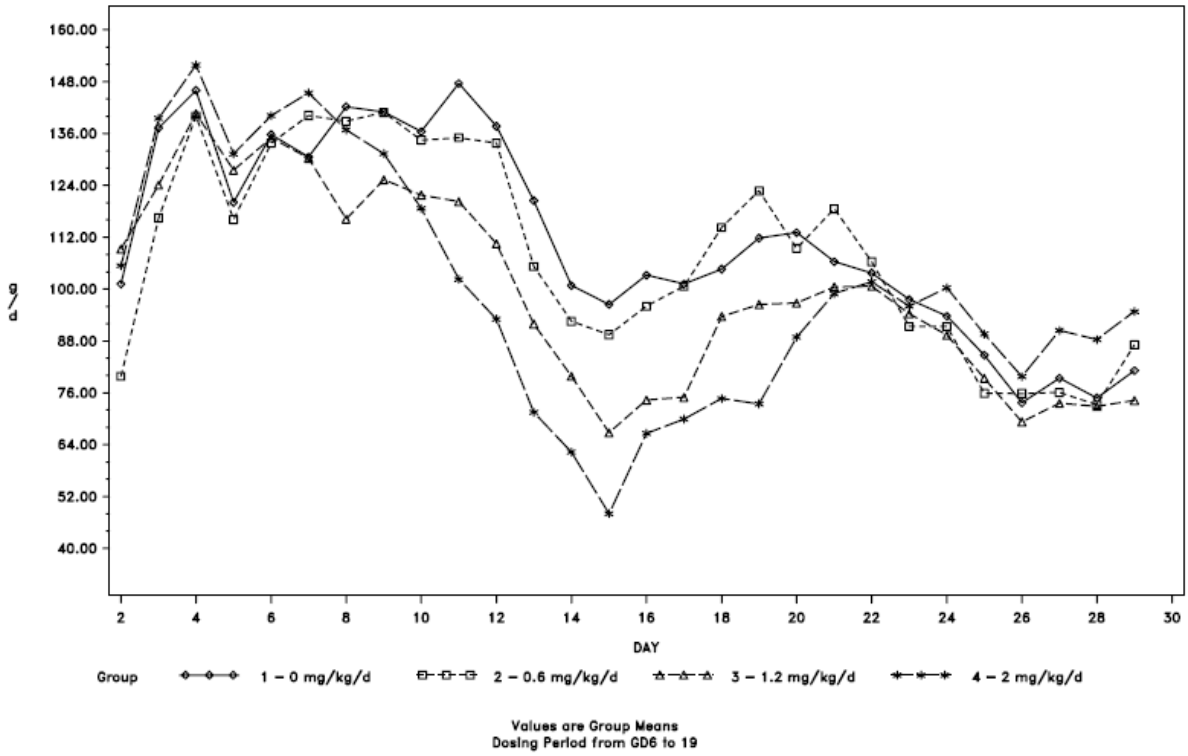


Figure 49. Pregnant rabbit group mean food intake during the gestation period

Toxicokinetics: All animals were found pregnant. The systemic exposures (Cmax and AUC) increased in proportion with the dose administered. Interconversion of MYK-461 to its enantiomer, MYK-460, after repeat-dose administration was quantified at low concentration levels especially at the high dose level. Both MYK-461 and MYK-460 peaked between 0.5 and 1 h post dosing (Table 125).

Table 125. MYK-461 and MYK-460 toxicokinetic parameters in plasma on GD12

Dose level (mg/kg/d)	MYK-461			MYK-460		
	t_{max}[a] (h)	C_{max}[b] (ng/mL)	AUC_{0-24}[b] (ng.h/mL)	t_{max}[a] (h)	C_{max}[b] (ng/mL)	AUC_{0-24}[b] (ng.h/mL)
0.6	0.50 [0.50-1.0]	516 ±152 (29)	7160 ±1430 (20)	NA	<LLOQ	NA
1.2	0.50 [0.50-1.0]	1100 ±55.1 (5)	16500 ±2000 (12)	NA	<LLOQ	NA
2.0	0.50 [0.50]	1680 ±191 (11)	29000 ±4210 (15)	1.0 [0.50-1.0]	0.406 ±0.0594 (15)	0.426 ±0.139 (33)

NA: not applicable ; LLOQ : 0.250 and 1.00 ng/mL for MYK-460 and MYK-461, respectively

a Median [min-max]

b Mean ±SD (CV%)

Additionally, MYK-461 was quantified in embryonic and extra-embryonic (fetal envelopes, placenta, amniotic fluid) tissues in pregnant animals, the day following the last administration (i.e., 24 h after dosing on GD 12). MYK-461 was distributed in theses tissues, with individual embryonic tissue to plasma concentration ratios ranging from: 0.09 to 0.15, 0.10 to 0.13 and 0.12 to 0.13 at 0.6, 1.2 and 2 mg/kg/day, respectively (Table 126).

Table 126. MYK-461: Individual embryonic and extra-embryonic tissues concentrations and embryonic tissue to plasma concentrations ratios

Dose (mg/kg/day)	Animal number	Embryonic tissue concentration (ng/g)	Extra-embryonic tissue concentration (ng/g)	Embryonic /Extra-embryonic tissue ratio	MYK-461 plasma concentration (ng/mL) [a]	Embryonic tissue / Plasma ratio
0	89	bql	bql	NA	<LLOQ	NA
	90	bql	bql	NA	<LLOQ	NA
	91	bql	bql	NA	<LLOQ	NA
0.6	92	26.2	70.3	0.4	276	0.09
	93	27.1	55.1	0.5	186	0.15
	94	22.3	49.2	0.5	172	0.13
	Mean	**25.2**	**58.2**	**0.5**	**211**	**0.12**
	SD	**2.55**	**10.9**	**0.1**	**56.4**	**0.03**
	%CV	**10%**	**19%**	**12%**	**27%**	-
1.2	95	54.1	114	0.5	546	0.10
	96	55	158	0.3	518	0.11
	97	53.4	91.1	0.6	425	0.13
	Mean	**54.2**	**121**	**0.5**	**496**	**0.11**
	SD	**0.80**	**34.0**	**0.2**	**63.3**	**0.01**
	%CV	**1%**	**28%**	**33%**	**13%**	-
2	98	118	192	0.6	929	0.13
	99	107	210	0.5	845	0.13
	100	143	256	0.6	1170	0.12
	Mean	**123**	**219**	**0.6**	**981**	**0.13**
	SD	**18.4**	**33.0**	**0.1**	**169**	**0.003**
	%CV	**15%**	**15%**	**10%**	**17%**	-

bql: below quantifiable limit; LLOQ: Lower Limit of Quantification

[a] Individual plasma concentration 24 hours after administration on GD12.

Necropsy: The terminal body weights with or without the uterus weight were comparable to control values at all dose levels. There were no treatment-related macroscopic observations except for those that died prematurely (Table 123).
Reproductive Performance: No effect of MYK-461 on the gravid uterine weight. The pregnancy rate, mean number of corpora lutea, implantations, post-implantation loss, number of live and dead embryos, and distribution of resorptions were similar across groups. Additionally, there was no effect of test substance on mean live fetal body weight and sex distribution across the groups (Table 127).

Table 127. Summary of female survival, pregnancy and cesarean section data

Dose levels, mg/kg/day	0 (Control)	0.6	1.2	2
Mated females	22	22	22	22
Unscheduled deaths	1	1	1	2
Surviving females	21	21	21	20
Abortion	1	1	1	0
Pregnant, surviving with live fetuses	20	19	21	20

Dose mg/kg/d		Corpora Lutea	-Preimplantation- Loss- Absolute (c)	Loss- %Corpora Lutea	Implant Sites (c)	Postimplantation Loss Resorptions Early	Resorptions Late	Dead Fetuses	Total (i)	%Implantations	Live Fetuses Absolute (i)	%Implantations	Total (i)	Live Fetuses Percent Males	Mean Live Fetal Wt (g) Male	Female	Total
Group 1	MEAN	12.4	1.7	13.9	10.7	0.5	0.1	0.0	0.60	5.3	10.1	94.7	10.1	46.6	30.92	30.96	30.99
0	STD	3.1	1.7	14.9	3.4	0.7	0.3	0.0	0.82	7.8	3.2	7.8	3.2	17.9	5.84	6.09	5.83
	MEDIAN	13.0	1.0	9.5	11.0	0.0	0.0	0.0	0.00	0.0	10.5	100.0	10.5	50.0	29.54	31.42	30.98
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Group 2	MEAN	12.5	2.3	17.5	10.2	0.6	0.2	0.0	0.79	8.9	9.4	91.1	9.4	48.7	32.55	30.63	32.00
0.6	STD	2.0	2.5	17.2	2.4	0.8	0.7	0.0	1.08	13.2	2.5	13.2	2.5	20.9	6.52	5.79	6.27
	MEDIAN	12.0	2.0	14.3	10.0	0.0	0.0	0.0	0.00	0.0	10.0	100.0	10.0	45.5	31.70	28.80	30.01
	% control	+1	+40	+26	-5	+16	+111		+32	+70	-7	-4	-7	+4	+5	-1	+3
	N	19	19	19	19	19	19	19	19	19	19	19	19	19	19	18	19
Group 3	MEAN	12.7	1.9	15.0	10.8	0.5	0.1	0.0	0.62	6.1	10.2	93.9	10.2	50.4	28.80	29.33	29.42
1.2	STD	2.4	1.6	13.5	2.6	0.7	0.4	0.0	0.80	8.3	2.8	8.3	2.8	15.7	5.65	4.69	4.36
	MEDIAN	13.0	2.0	12.5	10.0	0.0	0.0	0.0	0.00	0.0	10.0	100.0	10.0	54.5	29.47	29.22	29.06
	% control	+3	+15	+8	+1	-5	+43		+3	+17	+1	-1	+1	+8	-7	-5	-5
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Group 4	MEAN	11.7 NS	1.5 NS	12.1	10.2 NS	0.8	0.2	0.0	0.90 NS	8.7	9.3 NS	91.3	9.3 NS	55.7 NS	30.12 NS	29.15 NS	29.61 NS
2	STD	1.4	1.1	9.2	1.3	1.1	0.4	0.0	1.17	11.1	1.6	11.1	1.6	17.1	3.93	3.63	3.69
	MEDIAN	11.5	1.5	11.2	10.0	0.0	0.0	0.0	0.00	0.0	9.0	100.0	9.0	55.6	30.12	30.01	29.81
	% control	-6	-12	-13	-5	+50	+50		+50	+66	-8	-4	-8	+19	-3	-6	-4
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

* = Significant trend (p <= 0.05) through indicated dose level
NS = No significant trend through indicated dose level
NT = No test performed
i = Analysis stratified by number of implantation sites
c = Analysis stratified by number of corpora lutea

Fetal Anomalies: Test substance-related external, visceral and skeletal malformations were noted at ≥1.2 mg/kg/day (Table 128).

External Examination: A dose-related increased incidence of cleft palate was observed at ≥1.2 mg/kg/day. Cleft palate was recorded in 3 fetuses from 1 litter at 1.2 mg/kg/day and in 20 fetuses from 6 litters at 2 mg/kg/day (Table 125). Other external findings were of a type and occurrence comparable to that of the test facility historical control data.

Visceral Examination: Among visceral fetal observations, great vessels malformations (pulmonary trunk atresia associated with large ventricular chambers, dilated pulmonary trunk, and/or dilated aortic arch) were recorded in 4 fetuses from 4 litters at 2 mg/kg/day. Although one of these anomalies (dilated aortic arch) was noted in one control (polymalformed) fetus (associated with a ventricular septum defect), and at incidence encompassed by testing facility historical data, but these anomalies affecting great vessels (i.e., pulmonary trunk, and aortic arch) occurred at higher incidence at 2 mg/kg/day (3 fetuses in 3 litters) than at control. According to the sponsor, all other visceral malformations were observed in a control polymalformed fetus only or in treated fetuses at sporadic incidences (e.g., large kidney associated with dilated renal pelvis, small testis, and bilobed gallbladder) and were of a type and occurrence comparable to that of the test facility historical control data (Table 128).

Skeletal Examination: Test substance-related skeletal malformations were noted at ≥1.2 mg/kg/day. Increased incidence of sternebra malformation (i.e., fused sternebrae) was noted in 38 fetuses from 10 litters at 2 mg/kg/day and 12 fetuses from 5 litters at 1.2 mg/kg/day versus 6 fetuses from 4 control litters (Table 128). Rest of the skeletal malformations were considered unrelated to test substance administration as they also occurred in control fetuses, at low incidence, with no clear dose-related incidence, and/or were of a type and occurrence (rib and vertebra malformations) comparable to that of the test facility historical control data. Skeletal minor anomalies (variations, e.g., skull bone, rib, sternebra and ilium variation) were found in a few fetuses in all groups, including controls, and/or within the range of historical control data, and therefore, the sponsor considers them not related to MYK461 administration. Increased incidence of incomplete ossification (e.g., hyoid and metacarpal) relative to concurrent control was noted in all dose groups but with a slightly higher numbers in treated groups than in control. Some of these exceeded the historical control range for this strain (Table 128).

Table 128. Fetal anomalies- MYK-461-related external, visceral and skeletal malformations

Group		1	2	3	4
Dose level (mg/kg/day)		Control	0.6	1.2	2
Number of live fetuses (litter)		202 (20)	179 (19)	214 (21)	186 (20)
Morphology type	Class				
External					
Cleft palate	Malformation	0(0)	0(0)	3(1)	20(6)
Visceral					
pulmonary trunk atresia	Malformation	0(0)	0(0)	0(0)	1(1)
pulmonary trunk- dilated	Malformation	0(0)	0(0)	0(0)	1(1)
aortic arch –dilated	Malformation	0(0)	0(0)	0(0)	3(3)
ventricular chamber – enlarged	Malformation	0(0)	0(0)	0(0)	1(1)
ventricular chamber –small	Malformation	0(0)	0(0)	0(0)	1(1)
gall bladder – small	Minor anomaly	6(4)	7(2)	9(7)	10(3)
kidney - enlarged	Malformation	0(0)	0(0)	0(0)	1(1)
Skeletal					
Sternebra –fused	Malformation	6(4)	3(3)	12(5)	38(10)
Rib	Malformation	0(0)	1(1)	2(2)	1(1)
Hyoid incompletely ossified	Ossification	32(8)	41(12)	43(15)	35(14)
Metacarpal incompletely ossified	Ossification	34(8)	34(9)	34(13)	35(12)
Forepaw phalanx incompletely ossified	Ossification	29(10)	14(7)	33(11)	32(12)

9.4 Pre- and postnatal development toxicity study of MYK-461 in rats

Conducting laboratory and location: (b) (4)

Testing facility study number: 00384028
Sponsor study number: NC-18-0037
Date of study initiation: December 13, 2018
Date of termination: May 30, 2019
Drug lot/batch number: MYK-461, #180040, purity 99.42%
GLP compliance: Yes
QA statement: Yes, report signed

Key Study Findings

Oral administration of MYK-461 from GD 6 through lactation day 20 at doses up to 1.5 mg/kg/day in rats did not cause maternal deaths or decrease in body weight gain and food consumption. No effects of test article were observed on the reproductive performance of F0 maternal animals or F1 generation at any dosage level. There were no effects of MYK-461 on balanopreputial separation, vaginal patency, auditory startle response, motor activity, or learning and memory for F1 males and females. Based on the lack of adverse effects of MYK-461, the NOAEL for F0 maternal systemic toxicity, F1 neonatal/developmental toxicity, F1 parental systemic toxicity, F1 reproductive toxicity, and F2 embryonic toxicity was 1.5 mg/kg/day.

Purpose

This study was designed to determine the potential adverse effects of MYK-461 on exposure from implantation (Gestation Day 6) to weaning (LD 20) on pregnancy, parturition, and lactation of the maternal animals and on the growth, viability, and development of the F1 neonates. Also assessed the effects on the reproductive performance of the F1 generation.

Methods

Formulation

MYK-461 was suspended in aqueous 0.5% methylcellulose. Drug formulations were prepared every other week and refrigerated before use. It was stable for 2 weeks at room temperature or refrigerator conditions. Concentration and homogeneity of formulations prepared on the first, middle and the last batches were analyzed. Concentration results were considered acceptable if mean sample concentration results were within or equal to ± 15% of theoretical concentration.

Animals

Species/Strain: Rat (Crl:CD(SD); Sprague-Dawley)
#/Group: 24 pregnant females
Age: 10 to 13 weeks old when received
Weight: 205 to 246 g on GD 0
Husbandry: Pregnant F0 females were housed individually in cages. Following weaning F1 animals were group housed (3 animals of the same sex). During cohabitation, the subset F1 animals were paired for mating in the home cage of the male. Following the breeding period, the subset of F1 animals were individually housed. Animals were separated during designated procedures/activities. Food and water were given *ad libitum* throughout the study.

Dosing

Suspensions of MYK-461 were administered orally by gavage, once daily, to groups of presumed pregnant females at doses of 0.3, 0.75 or 1.5 mg/kg/day from GD 6 through lactation day 20 (Table 129). The F1 animals were not directly exposed to the test article at any time during the study; the offspring of the F0 parental generation were potentially exposed to the test article in utero and while nursing. The control animals received the vehicle (5 ml/kg body weight). The doses were selected based on a previous embryo-fetal study (Section 9.2) in the same rat strain in which MYK-461 was administered to pregnant rats from GD 6 through 17. In this study, developmental toxicity was observed at 1.5 mg/kg/day as evidenced by increased post-implantation loss, altered fetal growth (i.e., lower mean fetal body weight, and slightly reduced fetal skeletal ossification), visceral malformations (heart malformations in 2 fetuses, including 1 total situs inversus) and increased incidences of skeletal malformations (mainly fused sternebrae) relative to controls. The dosage levels from that study were repeated to determine if there are indirect reproductive effects in F1 generation other than teratogenesis.

Table 129. Study design

Group Number	Treatment	Dosage Level[a] (mg/kg/day)	Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Females
1	Vehicle	0	0	5	24
2	MYK-461	0.3	0.06	5	24
3	MYK-461	0.75	0.15	5	24
4	MYK-461	1.5	0.3	5	24

[a] No correction factor was used.

Observations and Measurements

F0 Generation (Maternal animals)

Clinical Signs: All F0 animals were observed twice daily throughout the study.

Body Weights: Individual body weights were recorded on gestation days 0 (before arrival) 5, 6, 7, 9, 12, 15, 18 and 21 and on lactation days 1, 4, 7, 11, 14, 18, and 21.
Food Consumption: Recorded on gestation days 5, 6, 7, 9, 12, 15, 18 and 21 and on lactation days 1, 4, 7, 11, 14, 18, and 21.
Parturition: All dams were allowed to deliver naturally and rear their offspring to weaning (post-natal day 21). The duration of gestation, litter size and the number of pups born (live and dead) were recorded.
Necropsy: All surviving F0 animals (n =24/group as there were no unscheduled deaths) with viable pups on lactation day 21 were euthanized. Females that failed to deliver were euthanized on post-mating Day 25. Females with total litter loss were euthanized within 24 h of litter loss. A gross necropsy was performed on each of these females which included examination of the thoracic, abdominal and pelvic cavities, including visceral contents. The number of former implantation sites was recorded for delivered females. The number of unaccounted-for sites was calculated for each female by subtracting the number of pups born from the number of former implantation sites observed. Number of corpora lutea was also recorded for females necropsied during gestation through Lactation Day 4. For females that failed to deliver, a pregnancy status was determined, and details that may have interfered with pregnancy was evaluated. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss. No histopathological examination was performed at necropsy and no tissues were retained from all F0 and F1 animals.

F1 generation

Viability: Litter size was monitored daily. Pups were observed for general health twice daily and detailed clinical observation was performed on PND 1, 4, 7, 10, 14, 17, and 21. Pups were individually sexed on PND 0, 4, 14, and 21.
Body Weights: Pups were individually weighed on PND 1, 4, 7, 10, 14, 17, and 21.
Offspring Allocation for Postnatal Evaluation: For the evaluation of attainment of developmental landmarks and neurobehavioral studies in F1 generation, 2 males and 2 females/litter were randomly selected prior to weaning. From these selected pups, 1 pup/sex/litter were assigned to Subset A, and the remaining male and female in each litter were assigned to Subset B (Table 130).
Necropsy: On PND 21, each non-selected F1 pup from all 4 groups was euthanized and subjected to a complete necropsy examination. All F1 pups not selected for breeding (Subset A) were euthanized following completion of the PND 62 learning, memory and other neurobehavioral assessment. All surviving

females were euthanized on GD 15 and the males were euthanized thereafter. F1 females with no evidence of mating were euthanized on post-cohabitation Day 15 (Table 131). All animals were subjected to a complete necropsy examination, which included examination of the thoracic, abdominal, and pelvic cavities, including contents. Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss. The ovaries and uterus were examined for number and distribution of corpora lutea, implantation sites, embryos, and early resorptions. Gross lesions were collected and preserved.

Table 130. Offspring allocation for developmental landmarks and breeding

Maximum No. Selected (Subset)	Age	Evaluation
24/sex/group (A) [a]	PND 20 and 60	Auditory Startle
24/sex/group (A) [a]	PND 21 and 61	Motor Activity
24/sex/group (B)	PND 22	Learning and Memory [b]
24/sex/group (A) [a]	PND 62	Learning and Memory [b]
24/sex/group (B)	Minimum of 85 days	Breeding

* For each endpoint, a maximum of 24 animals/sex/group were selected and represented 1 pup/sex/litter.
[a] The same pup subset was used for auditory startle, locomotor activity, and PND 62 learning and memory.
[b] Different pups were evaluated on PND 22 and 62.

Table 131. Terminal Procedures – F1 Generation

Group No.	No. of Animals M	No. of Animals F	Scheduled Euthanasia Day	Necropsy Procedures: Necropsy	Necropsy Procedures: Tissue Collection[a]
1	24	23	Not Selected for Breeding: PND 73–78	X	X
2	22	23			
3	22	23			
4	24	24			
1	24	24	Selected for Breeding: PND 123–128 (Males) Gestation Day 15 (Females)[b]	X	X
2	23	23			
3	22	23			
4	24	24			
Unscheduled Deaths				X	X

X = Procedure conducted.
[a] Gross lesions only.
[b] Females with no evidence of mating were euthanized on Postcohabitation Day 15.

Pups not selected for developmental landmarks and breeding were euthanized and necropsied on PND 21. Gross lesions were collected. The number of animals euthanized were 92, 87, 91 and 95 from groups 1, 2, 3, and 4, respectively.

Developmental studies (F1 generation): Twenty-four offspring of each sex were randomly selected from each group for developmental and reproductive studies.

The following development parameters were recorded for each pup at all dosage levels: balanopreputial separation, vaginal patency, auditory startle response, motor activity, and learning and memory.

Fertility Test (Breeding): At 12 weeks post-weaning (PND 85), F1 animals were cohabited (1 male and 1 female per litter but avoiding sibling mating) within the same treatment group for up to 21 days. Vaginal lavages were performed daily for 10 days prior to the initiation of the mating period and continued until evidence of mating was observed or until the end of the mating period. The day on which evidence of mating was identified was termed gestation day 0. Females with no evidence of mating were euthanized on post-cohabitation Day 15. Pregnant F1 females were housed individually until gestation day 15, when laparohysterectomies were performed. The ovaries and uteri were removed and examined for number and distribution of corpora lutea, implantation sites, embryos, and early resorptions. All males were euthanized subsequently.

Results

Analysis of Formulations: The achieved concentrations of MYK-461 for all doses were in the range from 83.3 to 101.6% of nominal.

Mortality: All F0 maternal animals in all dose groups including control survived to the scheduled necropsy.

Clinical Signs: No signs of toxicity were observed.

Body Weights: There were no effects of test substance on mean body weights and body weight gains either during gestation or lactation. Any small changes were considered to be within the expected biological variation.

Food Consumption: No effect of test substance during gestation or lactation.

Parturition: No effect of MYK-461 on mean duration of gestation. Pregnancy rates in treated and control groups were comparable (Table 132). Necropsy examination of F0 females sacrificed on lactation day 21 did not reveal any adverse effect of test substance.

F1 generation

The mean number of implantations and mean litter sizes were comparable among groups. The survival rates of pups from birth to PND 4 and from PND 4 to lactation day 21 were comparable in all groups. On PND 1, a small but statistically significant decrease in mean pup body weight in the high dose group relative to control was noted. On PND 21, the body weights were still lower than control but failed to attain statistically significant level (Table 133). The sponsor does not consider the low body weight of pups to be biologically relevant or related to test article administration. It is attributed to the higher mean number of pups born in the high dose group than in the control.

Table 132. Reproductive performance of F0 females

Parameters	Daily Dose (mg/kg/day)			
	0 (Control)	0.3	0.75	1.5
No. Evaluated	24	24	24	24
Clinical Observations	-	-	-	-
No. Died or Euthanized Moribund	0	0	0	0
No. Pregnant	24	24	23	24
Mean Duration of Gestation (days)	21.6	21.7	21.8	21.7
No. Aborted or with Total Res. of Litter	0	1	0	0
Abnormal Parturition	-	-	-	-
Gestation Body Weight g (%) Day 21	408	399 (-2.2)	394 (-3.4)	400 (-2.0)
Gestation Body Weight Gain, g (%) Day 21	152	142	138	146
Lactation Body Weight, g (%) Day 21	335	331 (-1.2)	325 (-3.0)	324 (-3.3)
Lactation Body Weight Gain (g) Day 21	35	32	36	33
Gestation Food Consumption Day 6-21, g (%)	23	22 (-4.3)	22 (-4.3)	22 (-4.3)
Lactation Food Consumption Day L1-21 g/animal/day	56	54	55	54
Necropsy Observations	-	-	-	-

- = no noteworthy findings

From PND 0 through the selection of pups, 3 (1), 6(3), 6(6), and 12(7) pups (litters) in the control, 0.3, 0.75, and 1.5 mg/kg/day groups, respectively, were found dead or euthanized in extremis. No internal findings that could be attributed to maternal administration of MYK-461 were noted at the necropsies of F1 pups that were found dead or euthanized in extremis. Visceral malformations of an intraventricular septal defect (1 mm in diameter opening in the anterior portion of the septum, an absent bicuspid valve, and a small tricuspid valve) was noted for Fetus #7763-01 in the 1.5 mg/kg/day group and situs inversus (the trachea, esophagus, heart, great and major vessels were laterally transposed) and lobular dysgenesis of the lungs (only 1 lobe present) were noted for Fetus No. 7811-01 in the 0.75 mg/kg/day group. No other internal findings were noted. No necropsy findings in male or female pups euthanized on PND 21 or following postnatal developmental landmarks and functional tests. All F1 animals survived to scheduled necropsy with no clinical findings that could be attributed to F0 test article administration.

Mean ages of attainment of balanopreputial separation and vaginal patency and mean body weights at the age of attainment were unaffected by F0 maternal test article administration. All developmental landmarks and functional test results were similar among the control and F1 animals in the treated groups. There were no effects on F1 mating indices, fertility indices or estrous cycles as a result of F0 test article administration. Intrauterine growth and survival of the F2 fetuses were

unaffected by F0 maternal treatment at all dose levels. There were no significant differences in mean pre- and post-implantation losses, numbers of corpora lutea, implantation sites and live F2 embryos between control and treated groups (Table 133). There were no test article-related gross necropsy findings noted at the F1 necropsies. In conclusion, the lack of adverse effects throughout the study, a dosage level of 1.5 mg/kg/day (the highest dosage level tested) was considered to be the no-observed-adverse-effect level for F0 maternal systemic toxicity, F1 neonatal/developmental toxicity, F1 parental systemic toxicity, F1 reproductive toxicity, and F2 embryonic toxicity when MYK-461 was administered orally (by gavage) to rats.

Table 133. F1 generation: post-lactation growth, development, behavior and reproductive performance

Parameters	Dose (mg/kg/day to F0 maternal females)			
F1 Litters: (Preweaning)	**0 (Control)**	**0.3**	**0.75**	**1.5**
No. Litters Evaluated	24	24	23	24
Mean No. of Implantations	13.3	13.4	13.7	14.4
Mean No. Pups/Litter	12.7	11.8	12.9	13.6
Mean No. Liveborn Pups/Litter	12.7	11.6	12.7	13.3
Postnatal Survival to Day 4 (%/Liter)	99.1	95.0	97.5	95.9
Postnatal Survival to Weaning (%/Liter)	99.5	100	99.5	99.5
Pup Body Weights g (%)				
Males				
PND 1	8.1	8.4 (3.7)	8.1 (0.0)	7.5 (-7.4)*
PND 21	56.0	57.6 (2.9)	57.9 (3.4)	55.2 (-1.4)
Females				
PND 1	7.7	7.9 (2.6)	7.6 (-1.3)	7.0 (-9.1)**
PND 21	54.8	56.7 (3.5)	55.7 (1.6)	52.8 (-3.6)
Pup Sex Ratios (% male/liter)	48.6	44.0	48.0	52.3
Pup Clinical Signs	-	-	-	-
Pup Necropsy Observations	-	-	-	-
F1 Males: (Postweaning) Post-lactation growth, development and behavior				
No. Evaluated Postweaning Per Litter	48	45	46	48
No. Died or Euthanized Moribund	0	0	1	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Body Weight g (%)				
PND84	545	543 (-0.4)	527 (-3.3)	517 (-5.1
PND 119	578	676 (-0.3)	656 (-3.2)	638 (-5.9)

Body Weight Gain g				
PND 21-84	489	485	468	462
PND 21-119	622	618	598	582
Developmental landmarks				
Mean Age at Preputial Separation (days)	43.3	43.0	42.5	43.5
Startle Response	-	-	-	-
Motor Activity	-	-	-	-
Learning and Memory	-	-	-	-
Breeding				
No. of Males that Mated	24	23	23	23
No. of Fertile Males	19	22	19	21
F_1 Females: (Postweaning) Post-lactation growth, development and behavior				
No. Evaluated Postweaning	48	46	46	48
No. Died or Sacrificed Moribund	1	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Premating Body Weight g (%)	299	310 (3.7)	296 (-1.0)	297 (-0.7)
Premating Body Weight Gain (g)				
PND 21-84	244	253	240	243
Gestation Body Weight g (%)	388	398 (2.6)	383 (-1.3)	384 (-1.0)
Gestation (G0-15) Body Wt Gain (g)	80	81	85	74
Developmental landmarks				
Mean Age of Vaginal Patency (days)	31.7	31.4	31.6	31.9
Startle Response	-	-	-	-
Motor Activity	-	-	-	-
Learning and Memory	-	-	-	-
Reproductive performance (subset B)				
Number of females on study	24	23	23	24
No. of Females that Mated	24	23	23	23
No. of Pregnant Females	19	22	19	21
Mean Precoital Interval	2.8	2.3	2.5	3.3
Mean No. Corpora Lutea	17.5	17.1	17.5	18.5
Mean No. Implantations	16.7	15.7	16.3	17.2
Mean % Preimplantation Loss	4.4	8.3	6.5	6.1
Viable embryos (Mean %)	96.4	94.2	91.7	95.3

- = no noteworthy findings

10 Special Studies

10.1 Three-month oral toxicity study in rats for MYK-461 impurity qualification

Conducting laboratory and location: (b) (4)

Sponsor Study number: NC-18-0036.00
Testing Facility Study Number: 8402265
Date of study initiation: January 28, 2019
Date of last necropsy: May 9, 2019

Drug, Lot number: a) MYK-461, 180040, 99.95% purity. MYK-460 level in drug substance is (b) (4)% (that is, (b) (4) µg/mg dose); b) MYK-461 impurity enriched batch, lot #DZ-00157-55, chiral purity 98.31% (contains MYK-460 = (b) (4)% (i.e., (b) (4) µg/mg dose), (b) (4) = (b) (4)% (i.e., (b) (4) µg/mg dose). Total impurities would be: (b) (4) mg/kg/day dose

GLP compliance: Yes
QA statement: Yes, signed

Key Study Findings

For the study, drug substance (MYK-461) was spiked with two impurities the inactive (R)-enantiomeric impurity MYK-460 ((b) (4)%) and (b) (4) ((b) (4)%). Daily oral administration of 0.3 mg/kg/day MYK-461 or impurity enriched MYK-461 did not result in unscheduled deaths. There were no changes in body weight, food consumption, and clinical and anatomic pathology relative to control animals. Statistically significant minimal to moderate increases in adrenal, heart, and spleen weights (absolute and relative to body weights) relative to control were noted in females administered MYK-461 or impurity enriched MYK-461 suggesting a test article related effect rather than an impurity-related effect. The effect lacked histopathologic correlation. Exposure (Cmax and AUC) was similar or minimally different between both lots on Day 91. The study did not measure plasma concentrations of spiked impurities. The NOAEL for the study was (b) (4) mg/kg/day for MYK-461 or impurity enriched MYK-461. In conclusion, no change in the toxicological profile of MYK-461 was observed as a result of spiking the test material with two additional impurities (MYK-460 and (b) (4)).

Purpose

The primary objective of the study was to evaluate the toxicity (not the toxicokinetic profile) and no observed adverse effect level of two different lots of test substance, MYK-461, of which one lot is spiked with additional concentrations of the impurities MYK-460 [the (R)-enantiomer] and (b) (4). Both lots were administered daily via oral gavage to rats for 13 weeks at 0.3 mg/kg/day MYK-461 or impurity enriched MYK-461.

Methods

Formulation

Test substances were individually suspended in 0.5% methylcellulose in distilled water. Drug formulations were prepared at least once every 2 weeks and refrigerated before use. Two sets of duplicate samples prepared for administration on day 1 and during weeks 4 and 13 of the dosing phase were evaluated for concentration.

Animals

Species/Strain: Rat, Sprague-Dawley Crl:CD(SD) from Charles River Labs
#/Sex/Group: 10 (Table 131)
Age: 6 to 7 weeks old at start of dosing
Weight: Males: 187 to 211 g; Females: 145 to 211 g at the start of dosing
Husbandry: Animals were housed in groups of 2 or 3 per cage. Food and water were available *ad libitum* throughout the study period except during the blood collection period for clinical pathology and necropsy where food was withdrawn.

Dosing

Doses: Animals were randomly assigned to 3 dose groups. Groups 2 and 3, respectively, received MYK-461- or impurity enriched MYK-461 (test article 2) at a dose of 0.3 mg/kg/day (Table 134).

Table 134. Study design

Group[a]	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration[b] (mg/mL)
	Males	Females		
1 (Control)	10	10	0	0
2 (Test Article 1)	10	10	0.3	0.06
3 (Test Article 2)	10	10	0.3	0.06

Test Article 1 was MYK-461; Test Article 2 was MYK-461 impurity enriched batch.
a Group 1 were administered vehicle control article only.
b Concentrations (Groups 2 and 3) were based on the test article as supplied. A correction factor was not used.

Impurity enriched MYK-461 (Test article 2) contained MYK-460 (b) (4)% ((b) (4)µg/mg) of pure drug substance plus spiked concentration (b) (4)% (i.e., (b) (4) µg/mg), (b) (4) = (b) (4)% (i.e. (b) (4) µg/mg dose). Total impurities would be: (b) (4)µg = (b) (4) impurity

Mode and Duration of Administration: Once daily orally by gavage for all 3 groups for 13 weeks. Control animals received drug vehicle (5 ml/kg).

Observations and Measurements

The in-life procedures, observations, and measurements were performed for all group animals. This included clinical signs, body weights, food and water consumption, ophthalmology, clinical pathology parameters (hematology, blood coagulation, clinical chemistry, and urinalysis), gross necropsy findings, organ

weights and histopathology examinations. For TK parameters, blood samples were collected from a jugular vein of all animals at 0.5, 1, 2, 4, 8 and 24 h after dosing on the study day 91 (3 to 4 rats/sex/time point).

Results

Analysis of Formulations: The achieved concentrations of MYK-461 (for both batches) tested from Day 1 and Week 4 were within ± 15% of target concentration. On the other hand, the preparation for Week 13 of test substance/article 1 was 60% and of test substance 2 (impurity enriched MYK-461) was 95%. Based on the results, the test article 1 was prepared again and was determined to be at the low end of the acceptable range (84 to 86.8%). The initial preparation for Week 13 at 60% of target concentration was used to dose animals between Days 85 and 87 before the second preparation was administered between Days 88 and 91 of the dosing phase. The TK series for Week 13 was based on the day 91 dose.

Mortality: All animals survived to their scheduled euthanasia on day 22.

Clinical Signs: No test substance-related clinical signs were noted in any animals.

Body Weights: A modest weight gain (5 to 10%) relative to control was noted in both treated groups. The changes were comparable between treated groups suggesting a MYK-461-related effect rather than an impurity-related effect.

Food Consumption: A statistically significantly increase (up to 26% relative to control) in food consumption was noted in both treatment groups. The changes correlated with an increase in body weight suggesting a MYK-461-related effect rather than an impurity-related effect.

Clinical Pathology: Any changes noted were of similar magnitude across groups and were considered incidental.

Organ Weights: Treatment-related minimal to moderately increase ($P \leq .05$) in adrenal, heart, and spleen weights (absolute and relative to body weights) relative to control were noted in females administered MYK-461 or impurity enriched MYK-461 suggesting a test article related effect rather than an impurity-related effect (Table 135). The effects lacked histopathologic correlation.

Macroscopic Observations: All macroscopic observations were spontaneous and occurred at a low or similar incidence across groups and thus considered not test substances related.

Table 135. Group mean organ weights (absolute and relative to body weight) in rats dosed with MYK-461 or impurity enriched MYK-461 for 13 weeks

	Sex	Males			Females	
	Test Article	NA	MYK-461	MYK-461 impurity enriched batch	NA	MYK-461	MYK-461 impurity enriched batch
	Dose Level (mg/kg/day)	0	0.3	0.3	0	0.3	0.3
Adrenal							
	Absolute Weight (g)	0.0600	107	99	0.0576	123*	123*
	Body Weight Ratio (%)	0.0107	106	98	0.0196	117	110
	Brain Weight Ratio (%)	2.5941	110	100	2.7902	123*	123*
Heart							
	Absolute Weight (g)	1.6430	98	103	0.9803	112*	112*
	Body Weight Ratio (%)	0.2952	97	101	0.3354	106	101
	Brain Weight Ratio (%)	70.9834	101	104	47.3749	111*	113*
Spleen							
	Absolute Weight (g)	1.1151	90	94	0.5654	107	115*
	Body Weight Ratio (%)	0.1997	90	92	0.1929	102	103
	Brain Weight Ratio (%)	48.3421	93	95	27.3275	107	116*

NA = Not applicable.
* = Statistically significant difference (absolute or relative) compared with respective control mean value.
Note: Values for absolute weight and ratio of organ weights (relative to body or brain) for test article-treated groups expressed as percentage control mean value.

Histopathology: No MYK-461- or impurity enriched MYK-461-related microscopic observations were noted.

Toxicokinetics: The toxicokinetic series for Week 13 was based on the Day-91 dose. The unintended low concentration preparation of Lot 1 (60% of target concentration, see above under 'Analysis of formulations') was only administered for a few days (Days 85 to 87) and was replaced with a new formulation that was determined to be at the low end of the acceptable range (84 to 86.8%). The new preparation was administered between Days 88 and 91 of the dosing phase. On Day 91, exposure was similar in males between the two lots (impurity spiked lot/non-impurity spiked lot) of MYK-461 with Cmax and AUC0-24 ratios of 0.953 and 0.973, respectively. However, exposure in females between the two lots of MYK-461 suggested minimal difference that may have been reflective of the lower than intended dose concentrations. In females Cmax and AUC0-24 ratios of 1.64 or 1.73, respectively, were noted. Since the systemic exposures between the two lots were similar or minimally different and only noted at on a single preparation, the sponsor suggests that this was considered not to have impacted the toxicological comparison of MYK-461 and impurity enriched MYK-461. The

plasma concentrations of impurities, especially MYK-460 in groups were not determined. Previous 6-week toxicity studies in rats and dogs have reported MYK-460 at a concentration of [(b) (4)]% of MYK-461. Peak concentration of MYK-461 was between 0.5 and 1 h post dosing. There was no sex-dependent difference in the systemic exposures of MYK-461 lots (Table 136).

Table 136. Summary of MYK-461 exposure data

Analyte	Day	Sex	Dose Level (mg/kg/day)	Dose Group	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-24} (h*ng/mL)
MYK-461	91	M	0.3	2	138	0.5	1550
				3	132	0.5	1510
		F	0.3	2	89.3	1.0	1280
				3	146	1.0	2210

Notes: Values are rounded to three significant figures (one decimal place for t_{max}).
AUC_{0-t} and AUC_{0-24} values were equivalent in all cases.
Group 2 was administered MYK-461, Lot 180040 and Group 3 was administered MYK-461 impurity enriched batch, Lot DZ-00157-55 [(b) (4)]

In conclusion, no change in the toxicological profile of MYK-461 was observed as a consequence of spiking the test material with two additional impurities (MYK-460 and [(b) (4)]).

10.2 Electrophysiological effects of acute and chronic exposure to MYK-461

The electrophysiological safety of MYK-461 (mavacamten) under both acute and sustained exposures is derived from in vivo electrocardiographic studies from pharmacodynamic and repeat dose toxicologic studies. Also, a few in vitro cell-based studies have deliberated the mechanisms related to QT interval prolongation and proarrhythmic potential of MVA. All the studies summarized here are discussed in detail in sections noted with each study.

A. Pharmacodynamic studies

1. Telemetry, Acute: Single 1.5 mg/kg, po, in dogs (NC-20-0059) (section 4.1.3.6)

MYK-461 demonstrated diastolic improvement properties on all measured hemodynamic and cardiac parameters. Test article produced marked reductions in systolic contractile indices (LVEF -35%, preload-recruitable stroke work PRSW, - 33%, myocardial stroke volume SV: -25%), and increased ventricular chamber dimensions (end-diastolic, EDV +16%) (Table 137). The functional inhibition of MYK-461 was exposure-dependent, since systolic function was inhibited to a lesser degree at 24 h than at 3 h after dosing (e.g., EF: -13 ± 2 vs. -28 ± 5% and dP/dtmax: -10 ± 3% vs. -28 ± 3%). MVA shortened both the absolute (mean) QT interval (215 vs 226 ms predose, $P < 0.05$) and the estimated QT interval at 1000 ms (QT1000: 225 vs 234 ms predose, $P < 0.05$) up to 24 h postdosing (Table 138). The inability of MVA to cause QT interval prolongation event at a supra- therapeutic dose of 1.5 mg/kg was probably linked to below threshold exposure reached at 3 h (254 ± 33 ng/mL) and at 24 h (147 ± 30 ng/mL) post dose relative to other studies (e.g., chronic toxicity studies) at comparable doses. This suggests that a low plasma concentration of MVA (not the dose) is sufficient to change cardiac indices (decreased EF and dP/dtmax and increased EDV and heart rate) but may not be sufficient to affect Ikr/hERG and late sodium current (see below under 'mechanistic studies'), which are implicated in QT interval prolongation. In contrast, the sponsor presents an argument that MVA prolonged the electromechanical window (EMw: 161 vs 113 ms predose, $P <0.05$), defined as the time interval between the end of electrical repolarization (Tend) and the onset of mechanical filling (Pmin) in the ventricle) resulting in either no change or decreasing QT interval (Fig. 50). Shortening of the EMw has been associated with known torsadogens, thus supporting a low proarrhythmic potential for MYK-461. This theory needs to be examined further as this is not a reliable parameter and varied in each experiment. [See Study #3 wherein there was no change in EMw; however, caused marked increase in QTc interval.]

Table 137. Acute hemodynamic and cardiac effects of MYK-461 in conscious dogs at 3 hr post dose (average of both studies)

	EF (%)	dP/dtmax (mmHg/s)	EDV (mL)	Ees (mmHg/mL)	PRSW (*mmHg)	SV (mL)	MBP (mmHg)	HR (bpm)
PRE	61.2 ± 1.9	3,295 ± 184	27.0 ± 0.7	8.7 ± 0.6	93 ± 4	17.6 ± 0.6	108 ± 4	99 ± 3
MAVA	40.1 ± 3.0	2,443 ± 180	30.8 ± 0.8	6.1 ± 0.5	63 ± 4	13.4 ± 1.1	110 ± 3	122 ± 4
Change %	*-35 ± 4↓*	*-27 ± 2↓*	*14 ± 2↑*	*-31 ± 3↓*	*-33 ± 2↓*	*-25 ± 5↓*	*3 ± 2*	*25 ± 3↑*

Conscious, healthy chronically instrumented dogs. Data are mean ± SEM, showing absolute values predosing/baseline (PRE) and 3 hours (MAVA) postdosing; changes (vs PRE) are shown in italics. ↑, ↓: $P < 0.05$ vs PRE. Mavacamten: 1.5 mg/kg PO (n = 16).
EF: left ventricular ejection fraction; dP/dtmax: peak rate of left ventricular pressure increases during systole; EDV: left ventricular end-diastolic volume; Ees, and PRSW: elopes of the end-systolic elastance and preload-recruitable stroke work (respectively), derived from end-systolic/diastolic pressure-volume relationships during brief preload reductions; SV: left ventricular stroke-volume (calculated); MBP: mean arterial (systemic) pressure; HR: heart rate.

Table 138. Acute electrocardiographic effects of mavacamten in healthy dogs

	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTcF (msc)	QT1000 (ms)	EMw (ms)	dP/dtmax (mmHg/s)	Exposure, Ct, ng/ml
PRE	91 ± 8	89 ± 4	43 ± 2	226 ± 9	261 ± 4	234 ± 7	113 ± 6	3189 ± 431	Not done
3 h	106 ± 7↑	88 ± 4	43 ± 2	215 ± 7↓	261 ± 5	225 ± 7↓	161 ± 15↑	2331 ± 298↓	254 ± 33
24 h	108 ± 10↑	89 ± 4	41 ± 1	214 ± 7	259 ± 3	226 ± 6	133 ± 7↑	2766 ± 258	147 ± 30

Dosing: MAVA, 1.5 mg/kg PO; n = 7; subjects were conscious and chronically instrumented. Data are mean ± SEM, showing absolute value both predosing/baseline (PRE) as well as at 3 hours and 24 hours postdosing; ↑, ↓: $P < 0.05$ vs PRE.
dP/dtmax: peak rate of left ventricular pressure increases during systole; EMw: electromechanical window, or time interval between the end of electrical repolarization (Tend) and the onset of mechanical filling (Pmin) in the left ventricle; HR: heart rate; MAVA: mavacamten; PO: oral; PR, QRS, and QT: duration of the electrocardiographic intervals reflecting atrio-ventricular (PR) and ventricular conduction (QRS), as well as repolarization (QT); QT1000: estimated QT interval duration at an RR interval of 1000 ms, from an exponential fit to the beat-to-beat QT and RR values; QTcF: rate-corrected (Fridericia) QT interval. Exposure, concentration measured at 3 and 24 h post dosing time points (Ct)

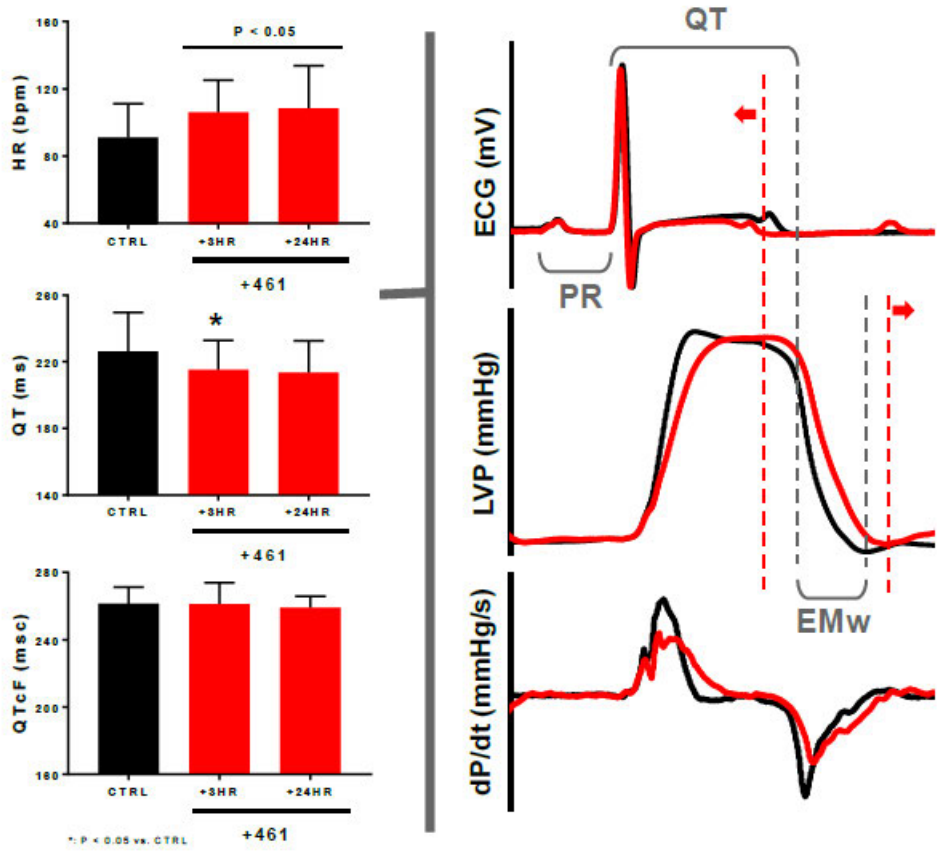


Figure 50. Acute electrocardiographic effects of mavacamten in conscious healthy dogs.
Data are mean ± SEM, before (PRE) and after mavacamten (MYK-461) administration (red).
*: P < 0.05 vs PRE.
In healthy conscious dogs, MAVA (1.5 mg/kg PO, n = 7) produced a modest downward shift of the beat-to-beat QT versus RR relationship but preserved rate-corrected QT intervals (QTcF) and lengthened the electro-mechanical window (EMw).

2. Telemetry, Acute: Single oral doses of 1, 3 and 10 mg/kg in dogs (NC-15-0007, Section 4.3.2 Safety Pharmacology)

Single oral doses of 3 and 10 mg/kg in dogs produced dose-related decreases in systolic (-23 mm Hg, 15%; and -39 mm Hg, 26% from the pre dose, respectively, peak 3-4 h post dose) and pulse pressure up to week 1 post dose. There was a concomitant compensatory dose-related increase in heart rate (+42 bpm, 47% and +119 bpm, 123% from the predose at 3 and 10 mg/kg, respectively, at 2 h post dose) up to Day 4. No appreciable increase in heart rate was observed on Day 8. At the 10 mg/kg dose, decreases in PR and QT intervals were observed up to Day 4. On Day 8, QT interval was statistically significantly increased on all hours of measurement. Heart rate corrected QT lengthening was observed on Day 1 and Day 7. On Day 1, prolongation of the QTc interval with a maximal change from predose of 18 msec at 2 to 4 h post dose, reached a peak of 22 msec at 24 h postdose. On Day 8, prolongation of QTc interval

was statistically significantly high on most of the hours of measurement with a maximal change from predose of 30 msec (Fig. 51). The time periods of increased QTc interval were concurrent with increased heart rate and decreased QT interval on Day 1 and unchanged heart rate and increased QT interval on Day 8 (Fig. 51). The sponsor attributing an increase in QTc interval to an increase in heart rate is not correct based on Day 8 data. Also, the plasma concentration of 10 mg/kg MVA on day 8 was 188 ng/ml vs 540 ng/ml (729 ng/ml determined on the day of telemetry) on Day 1.

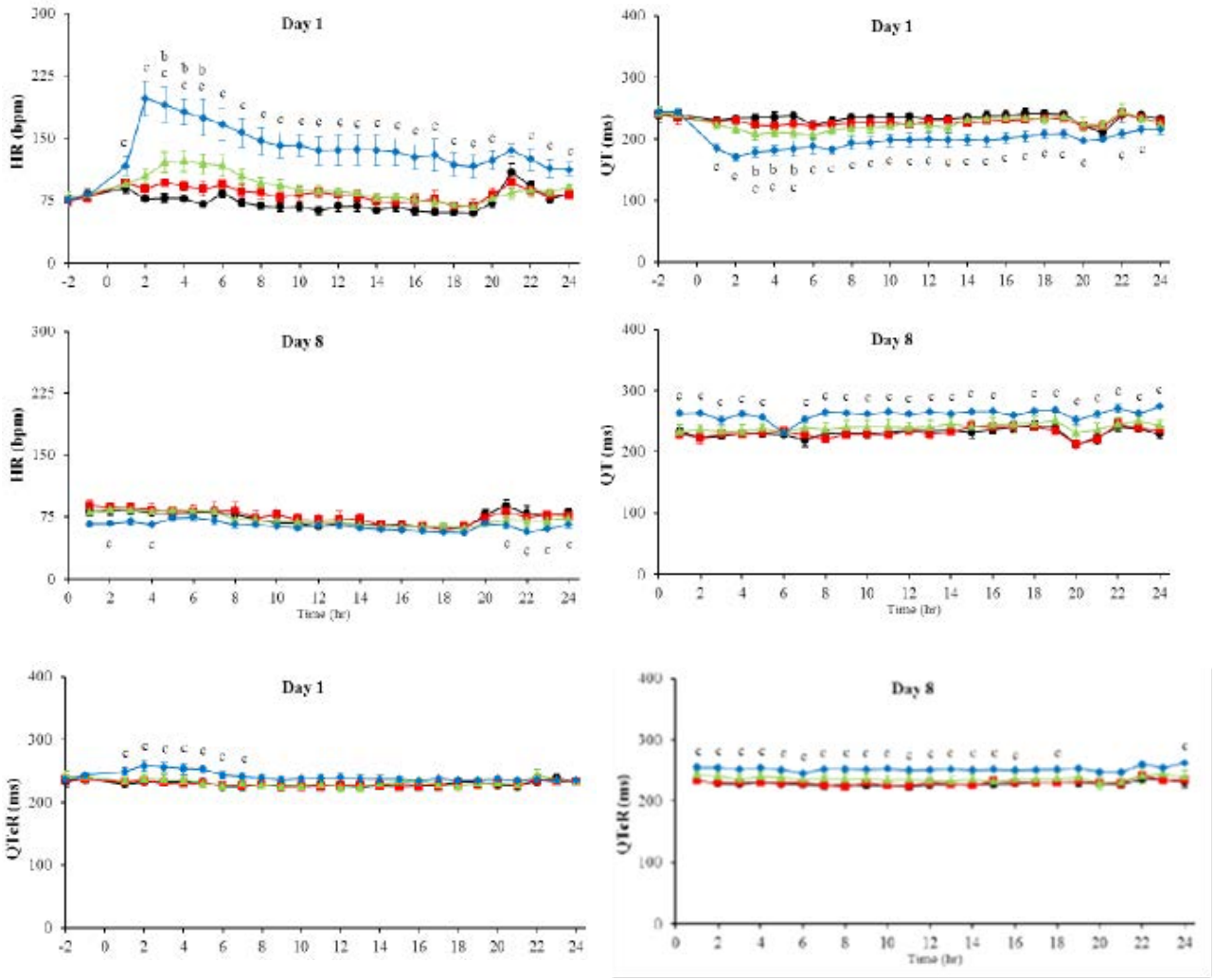


Figure 51. The effect of MYK-461 on heart rate, QT interval and heart rate corrected QT interval. Black: Control, Red: 1 mg/kg, Green: 3 mg/kg, Blue: 10 mg/kg. An 'a', 'b' or 'c' indicates that the mean value of the 1, 3 or 10 mg/kg group, respectively, is statistically different from the mean value of the vehicle control group. The plasma concentration of 10 mg/kg MVA on Day 8 Vs Day 1 was 188 vs 540 ng/ml (729 ng/ml determined on the day of telemetry).

3. Telemetry, Chronic study in dogs: 1.5 mg/kg twice on Day 1, followed by 12 doses of 0.3 mg/kg/day (NC-20-0060, see section 4.3.3)

As in the previous study #1, MVA demonstrated diastolic improvement properties on all measured cardiac parameters. MVA showed marked reductions in fractional shortening (on Day 15, EF: -26% and dP/dtmax: -22%, both $P < 0.05$ vs before treatment) and increased LV end-diastolic volume (EDV: +24%, $P < 0.05$). Concomitantly, upward shifts of the beat-to-beat QT versus RR relationship and QT prolongation were observed with QTcF prolongation reaching +17 ± 2 ms (273 ± 8 vs 255 ± 6 ms predose, $P < 0.05$) on Day 8 (plasma concentration, 415 ± 48 ng/mL) and +19 ± 2 ms (274 ± 8 vs 255 ± 6 ms predose, $P < 0.05$) at the end of the study (Day 15) (plasma concentration, 426 ± 54 ng/mL) (Table 139). QTcF prolongation is correlated (non-linearly) to EDV increase ($R2 = 0.6715$) (Fig. 52). According to the sponsor, QT prolongation was driven primarily to lengthening of the JTp (on Day 15, +19 ± 4 ms, 162 ± 5 vs 144 ± 4 ms at predose, $P < 0.05$), reflecting early repolarization (J-wave) changes, with negligible changes observed in the terminal portion of repolarization (TPE, on Day 15: +3 ± 2 ms, 43.0 ± 2.5 vs 40.4 ± 3.0 ms predose, $P > 0.05$) (Fig. 36 in Section 4.3.3). This suggests preserved transmural repolarization and unchanged late repolarizing (e.g., hERG) currents. No other notable electrocardiographic changes were observed postdosing as both QRS and PR intervals as well as heart rate and the length of the electromechanical window remained unchanged. [The length of the EMw was increased in Study #1.] In summary, QT prolongation was observed with QTcF prolongation ($P < 0.05$ on all days) with mild increase in heart rate, no changes in EMw, and submaximal plasma concentration of drug. The prolongation is correlated to increase in EDV (see also Study #4). This suggests that prolongation of QT/QTc is not dependent upon high plasma concentration of drug, heart rate or changes in the length of the EMw.

Table 139. Chronic electrocardiographic and mechanical effects of MYK-461 in healthy dogs (14-day repeat-dose study)

	Mechanical			Electrocardiographic							
	EF (%)	EDV (mL)	dP/dt_{max} (mmHg/s)	HR (bpm)	PR (ms)	QRS (ms)	QT (ms)	QTcF (ms)	JTp (ms)	Tpe (ms)	EMw (ms)
PRE	71 ± 2	40.5 ± 6.4	2,584 ± 185	77 ± 3	98 ± 4	45 ± 3	230 ± 6	255 ± 6	144 ± 4	40 ± 3	84 ± 5
Day 8	57 ± 2	46.5 ± 6.9	1,957 ± 136	80 ± 2	90 ± 3	43 ± 2	243 ± 8	273 ± 8	159 ± 7	43 ± 3	88 ± 5
Change	*-21 ± 4%↓*	*+15 ± 1%↑*	*-24 ± 4%↓*	*+5 ± 5%*	*-8 ± 3%*	*-4 ± 2%*	*+5 ± 2%*	*+17 ± 2↑*	*+15 ± 5*	*+3 ± 1*	*+6 ± 2%*
Day 15	53 ± 3	49.7 ± 6.4	1,987 ± 93	78 ± 3	91 ± 4	43 ± 2	247 ± 9	274 ± 8	162 ± 5	43 ± 2	89 ± 6
Change	*-26 ± 2%↓*	*+24 ± 3%↑*	*-22 ± 4%↓*	*+3 ± 7%*	*-7 ± 3%*	*-3 ± 2%*	*+7 ± 2%*	*+19 ± 2↑*	*+19 ± 4↑*	*+3 ± 2*	*+7 ± 4%*

dP/dt_{max}: peak rate of left ventricular pressure increase during systole; EDV: left ventricular end-diastolic volume; EF: left ventricular ejection fraction; EMw: electromechanical window, or time interval between the end of electrical repolarization (Tend) and the onset of mechanical filling (Pmin) in the left ventricle; HR: heart rate; JTp and Tpe: early (JTp, interval from the J-point to the peak of the T-wave) and terminal (Tpe, interval from the peak of the T-wave to its end); PO: oral; PR, QRS, and QT: duration of the electrocardiographic intervals reflecting atrioventricular (PR) and ventricular conduction (QRS), as well as repolarization (QT); QTcF: rate-corrected (Fridericia) QT interval.
↑, ↓: $P < 0.05$ vs PRE.
Dosing: 1.5 mg/kg twice daily on Day 1, 0.3 mg/kg/day on Days 2 to 15 PO; n = 4. Subjects were healthy, conscious, and continuously telemetered. Data are mean ± SEM, showing absolute value both predosing/baseline (PRE) as well as after 7 (Day 8) and 14 days (Day 15) of treatment; changes (from PRE) are shown in *italics*.
Total mavacamten exposures (in plasma): 415 ± 48 (Day 8) and 426 ± 54 ng/mL (Day 15).

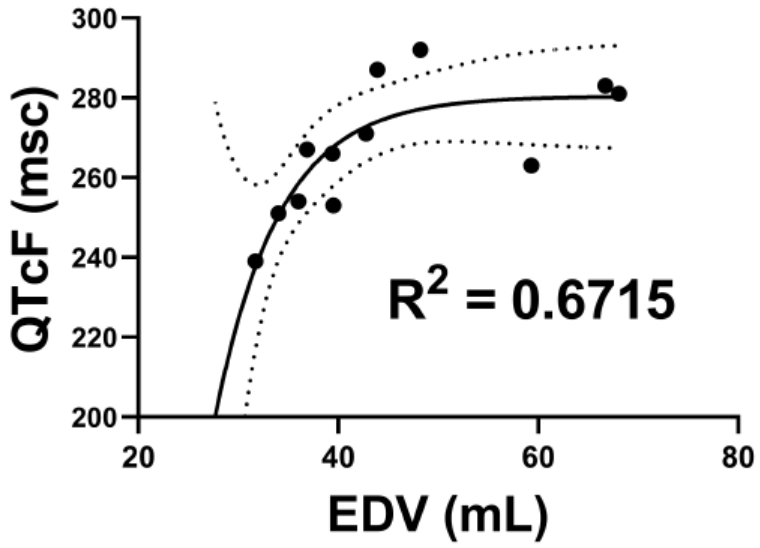


Figure 52. Relationship between QTcF and End-Diastolic Volumes (EDV) before/during sustained mavacamten exposure in healthy dogs.
Marked increases in left-ventricular end-diastolic volumes (EDV) was accompanied by heart rate-corrected QTc interval prolongations.

4. Chronic study in rats, 7-day repeat dose study (Study #NC-20-0062).

Male Sprague-Dawley rats (300 to 600 g) received daily oral doses of MYK-461 (2 to 4 mg/kg/day) for 7 days. Animals enrolled in the first cohort (Cohort A), received a single-dose of MYK-461 at 4 mg/kg/day for 7 days. Animals in the second cohort (Cohort B) received either a single-dose of MYK-461 (2 to 4 mg/kg, n = 12 with only one rat receiving the lower dose) for 7 days or served as time-controls receiving volume-matched vehicle. Cardiac function, structure (via high-resolution echocardiography) and ECGs were evaluated both prior to dosing (i.e., at baseline) and after 7 days of

treatment (approximately 3 h post-dosing). Blood samples were collected terminally on Day 8 via cardiac puncture.

Plasma concentration of MVA on day 8 was 902 ± 132 ng/mL at 4 mg/kg/day and 623 ± 55 ng/mL in animals receiving 2 or 4 mg/kg/day (details are not provided in the report).

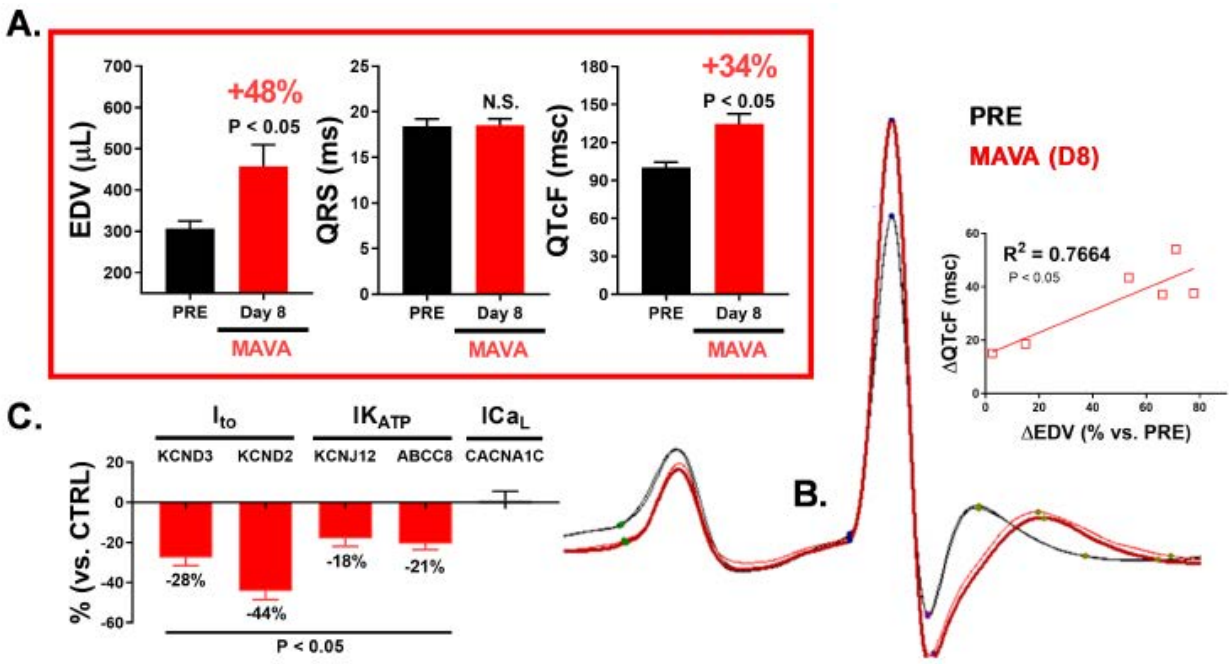
MYK-461-treated rats showed marked reductions in FS (-65 ± 4% vs -1 ± 4% in control, $P < 0.05$) which were accompanied by increased EDV (+48 ± 7% vs. +6 ± 3% in control, $P < 0.05$) and increased heart rate (+11 ± 2%, from 342 ± 7 to 379 ± 10 bpm, $P < 0.05$). In addition, rats showed significant ($P < 0.05$) prolongations of both the absolute (QT: +27 ± 6% or +15 ± 3 ms) and the rate-corrected QT interval (e.g., QTcF: +34 ± 6% or 34 ± 6 msc) (Table 140); QTcF changes were linearly predicted by the changes in EDV ($R2 = 0.77$, $P < 0.05$; Fig. 52 inset) and/or FS ($R2 = 0.79$, $P < 0.05$). No changes in PR and/or QRS intervals were noted, indicating preserved cardiac conduction.

The QT and mechanical changes were accompanied by downregulation of genes encoding the Ito (transient-outward potassium current that mediates repolarization) (KCND3/KCND2) and IK_{ATP} currents (KCNJ12/ABCC8 and KCNJ8). However, genes expression of calcium-handling (CACNA1C, RYR2, ATP2A2) and sodium-current (SCN5A) were unchanged.

Table 140. Mechanical and electrocardiographic effects of sustained mavacamten exposure (7-day repeat dose study) in conscious rats and effects on early repolarizing currents

	Mechanical (n = 18)			Electrocardiographic (n = 6)				
	FS (%)	HR (bpm)	EDV (µL)	PR (ms)	QRS (ms)	QT (ms)	QTcB (ms)	QTcF (ms)
PRE	47 ± 1	342 ± 7	330 ± 13	47 ± 1	18 ± 1	55 ± 2	135 ± 6	100 ± 4
Day 8	17 ± 2	379 ± 10	482 ± 23	45 ± 1	19 ± 1	71 ± 5	186 ± 11	135 ± 8
Change	*-65 ± 4%↓*	*+11 ± 2%↑*	*+48 ± 7%↑*	*-3 ± 2%*	*+1 ± 2%*	*+15 ± 3↑*	*+51 ± 8↑*	*+34 ± 6↑*
Gene Expression (vs CTRL) (n = 12)				*↓KCND3 (-28%), ↓KCND2 (-44%), ↓ABCC8 (-21%), KCNJ8 (-17%), KCNJ12 (18%),*				
				→CACNA1C (1%), →RYR2 (4%), ↓ATP2A2 (-14%), →SCN5A (-2%),				

EDV: left ventricular end-diastolic volume; FS: left ventricular fractional shortening; HR and RR: heart rate and interbeat interval; PO: oral; PR, QRS, and QT: duration of the electrocardiographic intervals reflecting atrioventricular (PR) and ventricular conduction (QRS), as well as repolarization (QT); QTcB and QTcF: rate-corrected (Bazett and Fridericia, respectively) QT interval.
↑, ↓: $P < 0.05$ vs PRE or CTRL.
Data are mean ± SEM, showing absolute value both predosing/baseline (PRE) and after 7 days of treatment (Day 8) with either placebo (CTRL, n = 12) or mavacamten (at 2-4 mg/kg/day PO); changes (from PRE) are shown in *italics*.
Total mavacamten exposures (in plasma): 902 ± 132 ng/mL (at Day 8).



Panels A and B: Sustained mavacamten exposure (MAVA, 2 to 4 mg/kg/day PO for 7 days) in healthy Sprague Dawley rats triggered marked function depression and ventricular enlargement (increased end-diastolic volumes, EDV) that were accompanied (correlated) by rate-corrected QT interval prolongations (Fridericia, QTcF). *Panel C:* QT changes were accompanied by downregulation of genes encoding the early repolarization currents, such as Ito (KCND3/KCND2) and IK_{ATP} currents (KCNJ12/ABCC8 and KCNJ8), when compared with vehicle-treated controls (CTRL).

ICaL: L-type calcium (Ca^{2+}) channel current; IKatp: adenosine triphosphate–dependent current; Ito: transient outward current; N.S.: not significant; PO: per os, oral; PRE: predose.

Figure 53. Mechanical and electrocardiographic effects of sustained mavacamten exposure (7-day repeat-dose study) in conscious rats and on genes encoding the early repolarization currents

B. Toxicology studies

1. 3-Month study in dogs (NC-19-0051, see section 6.2.7)

Doses: 0.06, 0.18, 0.30 and 0.45 mg/kg/day for 13 weeks except for the males receiving 0.45 mg/kg/day received the drug for 73 days.

A marked increase in heart rate relative to pretest values was noted in early decedents and surviving high dose group (0.45 mg/kg/day) animals and a female receiving 0.18 mg/kg/day. Slight QT, QTcv and QTcf prolongations were recorded in 2/6 females (51F and 52F) at 0.45 mg/kg/day (up to +8%, +7% and +9% in QT, QTcv and QTcf respectively, when compared with respective pre-treatment values), in 2/6 males (29M and 32M) at 0.3 mg/kg/day (up to +11%, +17% and +19% in QT, QTcv and QTcf respectively, when compared with respective pre-treatment values) and in 1/4 males

(21M) at 0.18 mg/kg/day (+13%, +14% and +16% in QT, QTcv and QTcf respectively, when compared with respective pre-treatment values) on Day 73 (group #5), Day 77 (males from Groups 1, 2, 3 and 4), Day 75 (females from Groups 1, 2, 3, 4 and 5), without any dose-relationship (Table 141).

Table 141. Heart rate and QT intervals after 73 or 86 doses of MYK-461 in dogs

Study Day	Heart Rate (bpm)		QT (milliseconds)		QTcf (milliseconds)	
	Male	Female	Male	Female	Male	Female
Group 1, Control	100	114	214	204	255	254
Group 2, 0.06 mg/kg	103 (+3)	102 (-11)	205 (-4)	211 (+3)	248 (-3)	255 (-)
Group 3, 0.18 mg/kg	90 (-10)	108 (-5)	222 (+4)	204 (-)	257 (+1)	247 (-3)
Group 4, 0.30 mg/kg	102 (+2)	106 (-7)	214 (-)	217 (+6)	258 (+1)	264 (+4)
Group 5, 0.45 mg/kg	132 (+32)	116 (+2)	201 (-6)	223 (+9)	260 (+2)	272* (+7)

*p<0.05, () = % different from control

0.3 mg/kg/day: Cmax: 496 M, 641 F ng/ml; AUC 7620 M, 9550 F ng.h/ml (on Day 86)
0.45 mg/kg/day: Cmax: 1040 M, 782 F ng/ml; AUC 17200 M, 12300 F ng.h/ml (on Day 73 for males and Day 86 for females). For details on TK, see Table 89.

2. 39-week study in dogs (NC-16-0048, see section 6.2.8)

Doses: 0.06, 0.18, 0.30 (Males) and 0.45 (Females) mg/kg/day for 39 weeks.

MYK-461 markedly (P <0.05) prolonged (+11 to +35 msec) QTcf interval in animals receiving ≥0.18 mg/kg/day relative to control and predose phase on Day 267 of the dosing phase (Table 142). The prolonged intervals were not observed on day 26 of the recovery phase in 50% of the high dose males and females. On an individual animal level, on Day 267 of the dosing phase, 3 of 4 males and 3 of 4 females receiving 0.18 mg/kg/day, all high dose males and females demonstrated longer (P <0.05) QTcf intervals than was on day 8 of the predose phase. Mean QRS duration was prolonged (+4 msec, 12%) in females receiving 0.45 mg/kg/day relative to control and predose phase. The prolongation was not observed in 50% of the high dose females on day 26 of the recovery phase. No rhythm abnormalities attributed to test substance administration was observed. A marked increase in heart rate relative to controls was noted in high dose males (+21 bpm, +24%) and in mid (+23 bpm, +29%) and high (+26 bpm, +33%) dose females on day 267 of the dosing phase (Table 142). A partial reversal in increase in heart rate was noted in high dose group animals on day 26 of the recovery phase. The NOAEL for QTc interval prolongation is 0.06 mg/kg/day

Table 142. Summary of QTcf interval (msec) and heart rate (bpm) data in males and females on day 267 (1 hr post-dose)

Group	Dose (mg/kg/day)	Statistics	QTcf		HR		TK Cmax D 273	QTcf		HR		TK Cmax D 273
			Pre-dose	D 267	Pre-dose	D 267		Pre-dose	D 267	Pre-dose	D 267	
			Males					Females				
1	0	Mean	236	234	78	87		245	234	90	80	
		SD	15.6	12.1	11.4	28.8		17.8	7.5	23.5	13.2	
		N	6	6	6	6		6	6	6	6	
2	0.06	Mean	251	244	100	81		240	234	93	94	
		SD	6.5	5.2	31.4	12.6		12.9	9.3	16.3	19.0	
		N	4	4	4	4		4	4	4	4	
3	0.18	Mean	246	257*	93	89	273	244	250*	92	103	300
		SD	5.4	15.9	18.3	19.8		15.7	10.9	15.1	18.7	
		N	4	4	4	4		4	4	4	4	
4	0.3 M/ 0.45 F	Mean	242	264*	93	108	445	241	269*	83	106	708
		SD	8.5	9.9	20.9	30.2		8.8	10.0	11.2	21.7	
		N	6	6	6	6		6	6	6	6	

* P ≤ 0.05. Cmax unit, ng/ml; D = Day. NOAEL for QTc interval prolongation is 0.06 mg/kg/day

Echocardiogram Examination: (See Section 6.2.8 for details). MYK-461 was associated with progressive and substantial increases in mean left ventricular end-diastolic (LVED) and mean LV end-systolic (LVES) volumes and a substantial reduction in mean LV ejection fraction in high dose males and females during the dosing phase relative to predose phase (Table 95, Fig. 44 in section 6.2.8). No echocardiographic effects were noted in 0.06 or 0.18 mg/kg/day groups. Test substance-related effects developed during weeks 23 and 37 of the dosing phase were reduced during Week 4 of the recovery phase although complete recovery was not documented (Table 95).

C. **Electrophysiologic in vitro studies**

1. Effect of MYK-461 on hERG channels expressed in HEK293 cells (Report #NC-14-0061) (Section 4.3.1)

MYK-461 reduced the amplitude of the outward tail currents by 5.0 ± 0.6% and 9.6 ± 0.6% at 10 and 60 µM, respectively. IC_{50} was not calculated, and on extrapolation it was greater than 60 µM.

2. Effect of MYK-461 on cardiac ion channels (Report #NC-19-0034/35) (Section 4.3.5).

The following ion channels expressed in HEK293 cells were evaluated: hCav1.2, hCav3.2, hHCN2, hHCN4, hERG, hKir2.1, hKir3.1/3.4, hKir6.2/SUR2A, hKv1.5, hKv4.3/KChiP2.2, hKvLQT1/hminK, and hNav1.5.
MYK-461 was evaluated up to a concentration of 30 µM. Inhibition was ≤ 6.9% and ≤12.6% at 10 and 30 µM, respectively, for all channels, with the exceptions of hKir6.2/SUR2A (-50.5 ± 25.1%) and hKv1.5 (-7.6 ± 4.2%).

MYK-461 inhibited hERG channel by 4.3 and 7.8% at 10 and 30 µM, respectively. Whereas MYK-1078 inhibited hERG channel by 4% at 30 µM.

3. Effect of MYK-461 on action potential parameters in isolated rabbit Purkinje fibers (Report #NC-19-0036/-0037) (Section 4.3.6).

MYK-461 at 30 µM (no effect up to 10 µM) induced a slight decrease in APD at 50% of repolarization (APD50: -4.3 ± 1.0%) with no effects on the resting membrane potential or other AP parameters, suggests a mild inhibition of Na+ and/or Ca^{2+} channels. Also, MYK-1078 (0.3 to 30 µM) had no statistically significant effect either on the resting membrane potential or on the action potential parameters.

4. Effect of MYK-461 on ion currents and action potential morphology in healthy and hypertrophied human primary ventricular myocytes (Report #NC-20-0061) (Section 4.3.7).

The effects of MYK-461 and its metabolite MYK-1078 were studied on individual ion currents, and on action potential morphology and duration in HEK293 cells and human ventricular myocytes isolated from either healthy or diseased hypertrophied patients. Furthermore, in vitro electrophysiological profile of MYK-461 (up to 30 µM) was studied in silico via action potential modeling in line with the CiPA (comprehensive in vitro proarrhythmia assay) (IKr, ICaL, INaL, IKs, IK1 and I_{to}) initiative to optimize for arrhythmogenic assessments using models for both healthy and hypertrophic myocytes. In addition, the potential for MYK-461 to induce QT prolongation in humans was evaluated via a proprietary QT fingerprinting in silico technique incorporating the effects on hERG, late-sodium (INaL), and L-type Ca2+ (Cav1.2, ICaL) currents.

Results

In human ventricular myocytes isolated from either healthy or hypertrophied hearts and expressed (HEK293) cells, MYK-461 concentration-dependently (0.3 to 30 µM) inhibited (-40.4 to 49%, IC50 30.7 µM) INa (late)/ Nav1.5 (late) current, and to a moderate extent (-5% to 31.5%, IC50 >30 µM) IKr/ hERG current. In the same setup, metabolite MYK-1078 was more efficacious/potent than the parent compound (*see* Table 24 in sec 4.3.7). MYK-1078 concentration-dependently (0.3 to 30 µM) inhibited (-20 to 82.4%, IC50 9.4 µM) INa (late)/ Nav1.5 (late) current, and to a greater extent (-28.9 to 74.2%, IC50 5.3 µM) IKr/ hERG current (*see* Fig. 37 in sec 4.3.7). MYK-1078 was 3.3- and 5-fold more potent than MYK-461 in blocking INa (late)/ Nav1.5 (late) and IKr currents. The blockade of IKr and INa (late) in myocytes from hypertrophied hearts was similar to that observed in myocytes from healthy hearts. On action potential duration, MYK-461 caused a concentration-dependent shortening (-25%) (*see* Fig. 38 in sec 4.3.7), while

MYK-1078 prolonged (+13.4%) the duration (*see* Fig. 39 in sec 4.3.7). According to the sponsor, the increased APD is probably the result of a greater blockade of INa (late) than IKr. The in-silico model predicted a small increase in APD under healthy conditions (+9.8% at 30 µM) and less of an increase under hypertrophied conditions (+3% at 30 µM). Under no conditions are there any indications of early afterdepolarizations (EADs). When INa and ICa are omitted from the model and there is just an outward current blockade, there is a much larger increase in APD and under some conditions EADs. Incorporating the effects on hERG, late-sodium (INaL), and L-type Ca2+ (Cav1.2, ICaL) currents, QT fingerprinting in silico technique predicted a lack of QT prolongation or a QTc shortening for MYK-461 and QTc prolongation for the metabolite MYK-1078.

Summary and Discussion

Prolongation of the QTcF was seen in both acute (single dose) pharmacodynamic studies at doses of ≥ 1.5 mg/kg in dogs; and repeat dose toxicity studies, 1-week to 39-Week at doses of ≥ 0.18 mg/kg/day in both rats and dogs (Summary Table 143). In acute pharmacodynamic studies, a biphasic ECG profile for MYK-461, characterized by preserved, shortened (up to 24 hours in one study) or prolonged QT interval as well as a late-onset QT/JTp prolongation was observed. These changes were noted with mild to moderate to profound increase in heart rate irrespective of changes in the length of the EMw. Prolongation of EMw in a single dose and a 14-day repeat dose studies in dogs suggest low proarrhythmogenic potential of MYK-461. QTc interval prolongation noted during post dosing days in single (on Day 8) or repeat doses (delay in onset) suggest a high plasma concentration of drug is not a prerequisite. On the other hand, QTc prolongation is always correlated to reduction in fractional shortening and increase in EDV. Taken together, a low plasma concentration of MVA (and not the dose) is sufficient to change cardiac indices (decreased EF and dP/dtmax and increased EDV and heart rate) as well as QT interval. According to the sponsor, delayed QT prolongation, though likely hERG independent suggests a low torsadogenic or proarrhythmic risk, occurs as adaptive mechano-electrical remodeling due to sustained concomitant functional and structural effects but could also be indicative of a direct effect of MYK-461 (or a metabolite) on ionic homeostasis, currents, or channel trafficking.

According to the sponsor, delayed QT/QTc interval prolongation observed after a single dose or sustained exposure in healthy animals (Table 143) is likely an adaptive response to sustained myosin inhibition in ventricles with normal physiology. Additionally, QT interval prolongation can be triggered by changes in LV mechanics and/or by increases in diastolic pressures via “mechano-electric” coupling processes and increase in heart rate. Thus, QT interval prolongation with MVA cannot be discussed in isolation but in context with LV mechanics (such as EDV, EDP), heart rate, the extent of exposure (Cmax, AUC) triggering late Na current.

Table 143. In vivo studies- pharmacodynamic (acute) and repeat dose toxicology

Study #/Rev section#	Description	Dose mg/kg	Cmax ng/ml	HR	QT	QTc	Remarks
NC-20-0059 /4.1.3.6	Acute, Single	1.5	3 h: 254 24 h: 147	+17% +19%	-5% -5%	No change	prolonged the electromechanical window; below threshold plasma conc.
NC-15-0007/ 4.3.2	Telemetry, Acute, Single dose	10	D1: 540-729 D8: 188	D1:+123% D8: NC	Decreased Increased	+18ms +30 ms	On D8, QTc increased on all hours of measurement.
NC-20-0060/ 4.3.3	Chronic, 14 doses of 0.3	1.5 twice on D1, followed by 0.3	D8: 415 D15: 426	+5% +3%	+5% +7%	+17 ms +19 ms	lengthening of the J-Tp on all days, +19 ms; +6% increase in EMw
NC-20-0062*/ 10.2.A4	Chronic 7-day in rats*	2 to 4	D8: 902	+11%	+27%	+34 ms 34%	linearly predicted by the changes in EDV, redn in FS; down regulation of genes-Ito
NC-19-0051 /6.2.7	13-week tox, 0.06 to 0.45	0.30 0.45	496-	+ mild increase	+6 ms +9 ms	+4 ms +7 ms	Determined on day 90 of dosing
NC-16-0048/ 6.2.8	39-week tox, 0.06, to 0.45	0.18 0.3M/0.45F	273-300 445M/708F	+29% +33%	NA	+11 to +35ms P<0.05	Determined on day 267 of dosing

All the studies were in dog, oral dosing, except for one indicted by asterisk was in rats (NC-20-0062).

Multiple in vitro electrophysiological studies were performed to elucidate both the specific mechanism(s) of the QT behavior and its torsadogenic risk (Table 144). Late sodium current (INa,L) is the major ionic current for rate adaption of repolarization. The most prominent effect of MYK-461 was a concentration-dependent shortening of APD. This is likely due to a greater blockade of INa (late) (IC50 30.7 µM) than IKr (-31.5% at 30 µM and IC50 could not be determined). MVA reduced INa,L current with more robust effects in diseased (hypertrophied) cells than healthy cells. Although metabolite MYK-1078 was more efficacious than parent compound with hERG (IC50, 5.3 µM) and late sodium (IC50 9.4 µM) current blocking with a modest increase in APD and proarrhythmic potential, it is unlikely to be the source of QT interval prolongation considering its negligible circulating levels (<0.2 µM total at Cmax). Furthermore, both MYK-461 and MYK-1078 had mild dose-dependent blocking effects on the Cav1.2 and Iks channels. Blocking late sodium current did not attenuate the mild blocking of hERG current and also failed to shorten the J-Tpeak or decrease EMw as both are associated with known torsadogens. However, this property of late sodium current blocking with a minor effect on hERG potassium channel can reduce arrhythmic events without

affecting therapeutic efficacy (reduction in FS, increase in EDV) as evident in all pharmacodynamic studies. Furthermore, inhibition of late sodium and calcium currents can prevent EADs as evident in APD studies and this has antiarrhythmic effects not leading to TdP. However, multichannel blockade (Johannesen et al) might contribute to MVA QT interval prolongation with a low torsadogenic or proarrhythmic risk. In conclusion, although QT interval prolongation is evident with the administration of MVA in dogs and rats, it is unlikely to precipitate arrhythmias and predicted to be minimal risk for torsade de pointes. In dogs, **the NOAEL for QTc interval prolongation is 0.06 mg/kg/day, which is 0.08 to 0.1 times human exposure (AUC) at the MRHD.**

Table 144. Mechanistic studies

Study #	Description	IKr/hERG		INa (late)/Nav1.5,L		APD	
		MVA	MYK-1078	MVA	MYK-1078	MVA	MYK-1078
NC-14-0061, Sec 4.3.1	Cloned hERG potassium channels expressed in HEK293 cells	-5.0 and -9.6 % at 10 and 60 µM					
NC-19-0034/35, Sec 4.3.5	hERG channels expressed in HEK293 cells, patch clamp system	-4.3 and -7.8% at 10 and 30 µM	-4% at 30 µM	-1.5 to -3% at 10 µM			
NC-19-0036/-0037Sec 4.3.6	Action potential parameters in isolated rabbit Purkinje fibers					At 30 µM APD90 -2.4%; APD50 -4.3%	No effect up to 30 µM
NC-20-0061 Sec 4.3.7	Ion currents and AP morphology in healthy and hypertrophied human primary ventricular myocytes and HEK	-31.5% at 30 µM IC50, ND (>30)	-74.2% at 30 µM IC50, 5.3 µM	-40 to -49% at 30 µM IC50 30.7 µM	-79.3 to -82.4% at 30 µM IC50 9.4 µM	APD90: -25% at 30 µM	+13.4% at 30 µM
	In silico prediction					APD90: +9.8% (healthy) and +3% (in hypertrophy)	

"-"means a decrease/reduction in current amplitude.

1 µM is 273 ng/ml total, free is 7%; death occurred at ≥3 µM = 820 ng/ml (free: 57 ng/ml). The circulating levels of MYK-1078 is negligible (<0.2 µM total at Cmax).

11 Integrated Summary and Safety Evaluation

A novel approach to suppress abnormally high left ventricle systolic function to relax (lusitropy) is through inhibition of cardiac myosin, the force-generating protein. This is achievable by modulating the number of myosin heads that can enter on actin states and thereby reducing the force of contraction. Based on this concept, mavacamten (MYK-461, MVA), a small molecular weight cardiac myosin inhibitor with negative inotropy and positive lusitropy properties was developed for the treatment of adults with symptomatic hypertrophic cardiomyopathy (HCM) to reduce left ventricular outflow tract obstruction and ventricular filling pressures. Mavacamten was evaluated for its potential inhibitory effect on cardiac contractile force in vitro and in vivo healthy animals, and in mice with pathogenic HCM mutations. Safety pharmacology studies were conducted to assess its safety in critical organ systems and torsadogenic proarrhythmogenic liabilities. Several in vitro and in vivo studies were performed to characterize its pharmacokinetics, protein binding, metabolite profile and the enzymes involved in its metabolism. Mavacamten has been tested in a complete range of animal toxicity studies, including carcinogenicity, genotoxicity and reproductive toxicity studies. The review describes these studies and evaluates their adequacy as support for the administration of mavacamten to obstructive HCM patients in accordance with the proposed product labeling.

Pharmacology

Biochemical studies measured interspecies sensitivity, steady state kinetics and the selectivity of MVA for cardiac versus skeletal forms of myosin. These studies suggest that MVA inhibits bovine myosin (IC50, 0.27 µM) more potently than human myosin (IC50, 0.39 µM). It is potent against both alpha- (rodents) and beta- (non-rodent) cardiac myosin. In rats, 5-fold selectivity exists between cardiac myofibrils and skeletal (fast/ slow) myofibrils. MVA inhibits recombinant human beta cardiac actomyosin and five HCM mutant myosins with comparable potencies suggesting the introduced mutations do not impact the inhibitory effect of MVA on myosin. Mavacamten is selective for cardiac and slow striated-muscle myofilaments (same myosin-isoform), and approximately 3-fold less selective for fast-twitch skeletal isoforms of myosin (rabbit, IC50 0.76 Vs. 2.14 µM) (Kawas et al. 2017). According to the sponsor, MVA has no impact on skeletal performance at exposures triggering marked reductions in LV function. Furthermore, MVA has no activity (IC50 > 50 µM) on smooth-muscle myosin-S1.

In a study probing the effect of MYK-461 on the release of ADP and Pi following ATP hydrolysis by ATPase, MYK-461 stabilizes an 'off-actin' conformation of myosin that slows the release of ADP. Inhibition of cardiac myofibrillar ATPase activity translates to decreased contractile force of the myocardium (fractional shortening of muscle), myofilament tension, and at the ensemble level, overall myocyte systolic function. MYK-461 decreases actin-activated Pi release rates in a concentration-dependent manner; with reported IC50 values of 1.85 ± 0.14 μM and 1.78 ± 0.07 μM in bovine and human cardiac myosin-S1, respectively. In adult rat ventricular myocytes, MYK-461 demonstrated a concentration-dependent inhibition of contraction with an IC50 of 200 nM. Calcium transients remained unchanged. During diastole, MYK-461 limits residual cross-bridge formation by decreasing the pool of myosin heads available to form strong cross-bridges as well as by stabilizing a low-energy utilization off-actin population of myosin known as the super relaxed state (SRX). This biophysical mechanistic study (a reduction in overall myocardial power) supports the efficacy of MYK-461 in oHCM.

In vivo studies were conducted in healthy mice, rats, and dogs in conscious and anesthetized state to determine the exposure-effect relationship of MYK-461 on cardiac contractility. In all species studied, MYK-461 induced myosin inhibition triggering both significant reductions in systolic contractile indices and increased ventricular chamber dimensions that were linearly predicted by circulating plasma concentrations. In rats, single oral doses of 1 to 10 mg/kg, with plasma concentrations ranging from 245 to 1019 ng/ml, produced 23% to 73% reductions in FS while markedly increasing EDV (11 to 28% from baseline). In conscious dogs, MYK-461 (1.5 mg/kg, PO) reduced dP/dtmax and fractional shortening (-27% at Cmax of 254 ng/ml), effectively slowing the velocity of contraction and prolonging the pre-ejection period (contraction time: +25%), while also decreasing end-systolic elastance (-31%), preload-recruitable stroke work (-33%), and myocardial stroke-work (-25%), indicating a reduction ($P < 0.05$) in overall myocardial power. Hemodynamic measurements showed preserved mean arterial pressures with dose-dependent reductions in arterial pulse pressure, cardiac output and mild to moderate concomitant cardio-acceleration. Similar observations (depressed systolic function with preserved diastole) were noted in dogs receiving an acute intravenous dose of 1.2 mg/kg. At 3.6 mg/kg IV, there was a greater reduction in LV systolic function (EF: -53%) along with a decrease in systolic blood pressure, marked cardio-acceleration and a reduction in cardiac output. The results from chronic low oral dose (45 μg/kg/day for 31 days) were more gradual in onset and lesser in magnitude (EF, -11% vs -20%) than the normal oral or IV dose. The cardiac effects started 4 days after treatment and peaked and stabilized after 25 days. Also, the plasma concentrations of MYK-461 steadily increased over time, reaching steady-state on day 19. In mice with pathogenic HCM mutations in Troponin T (R92L and R92W) presenting hyperdynamic contraction and impaired relaxation (diastolic dysfunction) with minimal

fibrosis, chronic MYK-461 treatment improved relaxation while preserving fractional shortening.

Two inotropes from two different classes (dobutamine and levosimendan) counteracted MVA-mediated inhibition of LV contractility in conscious rats and dogs and in isolated adult rat ventricular myocytes in vitro. MVA blunted β–AR-induced elevations in dP/dtmax and Vmax, indicating preserved contractile attenuation even in the setting of β-AR stimulation. The reversal was transient because the long-lasting effect of MVA overcame the short-lived action of dobutamine.

The lusitropic (ability to relax following excitation contraction coupling) effects of MVA were sustained in isoproterenol-treated cardiomyocytes by faster rates of re-lengthening and increased diastolic (resting) sarcomere lengths. Cardiomyocytes with functional inhibition by MVA maintained β-adrenergic signaling and the recruitability necessary for physiological cardiac compensation mechanisms. Addition of metoprolol in the presence of isoproterenol did not affect the cardiac negative inotropy with concomitant positive lusitropy of MVA.

Secondary Pharmacological screens confirmed MVA on-target selectivity that evaluated against a panel of kinases, enzymes and binding receptors. No significant effects (defined as ≥ 50% stimulation/inhibition) were noted with 10 µM MVA. No evidence of cytotoxicity was observed in the cell lines at concentrations up to 30 µM.

Safety Pharmacology studies showed that MVA has no impact on skeletal muscle performance or function in vivo (as there were no MVA-induced changes in forelimb/ hindlimb grip strength as well as locomotion activity relative to vehicle control) at exposures triggering marked reductions in LV function. Also, at the highest dose tested (10 mg/kg) in rats, respiratory effects were limited to transient increase in respiration rate and decrease in tidal volume. The electrophysiological safety of MVA under both acute and sustained exposures were established from multiple in vitro and in vivo studies in rats and dogs. These studies are described and discussed at length in a separate section (10.2). A greater blockade of INa (late) than IKr along with dose-dependent blocking effects on the Cav1.2 and Iks channels (multi-channel effect) suggest that MVA might trigger QT interval prolongation but with a low torsadogenic or proarrhythmic risk not leading to TdP.

Pharmacokinetics/ADME

Single oral and i.v. dose pharmacokinetic studies of MVA were investigated in mice, rats, dogs, and monkeys. Following oral dosing, peak plasma concentration reached between 0.3 and 0.7 h in all species. The mean half-life was shorter in mice (4.8 h) and rats (8.2 h) than in monkeys (43 h) and dogs (161 h). Oral bioavailability of MVA was high in all species studied (47 to >100%) and it depended on particles size. Following i.v. administration of MVA, concentration-time profiles demonstrated rapid distribution phase followed by biexponential decay and slow elimination phase. The mean volume of distribution at steady state was high in monkeys (10.6 L/kg), moderate in dogs (7 L/kg), and low in rats (5 L/kg) and mice (3.8 L/kg). The blood to plasma ratio was low suggesting a lack of distribution to erythrocytes. Consistent with high volume of distribution, elevated levels of MVA were noted in skeletal muscle followed by cardiac muscle in the rat and dog. Striated (cardiac and skeletal) muscle to plasma concentration ratio (>10:1) was higher than smooth muscle to plasma ratio (3:1). Low concentrations were observed in the CNS suggesting MVA is unlikely to cross the blood-brain barrier. MVA was moderately to highly bound to plasma proteins that ranged from 83.6% in the mouse to 97% in the monkey. The binding of MVA to human serum albumin was 92.9%. Test substance was cleared at a low rate, 2% of liver blood flow in the dog and ranged from 7 to 10% of liver blood flow in the mouse, rat and monkey. The elimination half-life (t1/2) was longer for dogs (130 h) than was for monkeys (44.5 h). It was relatively short for rats (11 h) and mice (7 h).

The primary mechanism of elimination is CYP-mediated oxidative metabolism. Several cytochrome P450 enzymes, including CYPs 2C19, 3A4/5, and 2C9 (74%, 18%, and 7.6%, respectively) are involved in the metabolism of MVA. Biotransformation studies of [14C]MYK-461 in microsomes and hepatocytes showed mice, rats, dogs, and monkeys metabolites are qualitatively and quantitatively similar to human. The primary metabolites observed in rat plasma were M1 (MYK-2210) and M2 (MYK-1078), which were the oxidative metabolites formed in all species including human liver microsomes. Other predominant metabolites identified across species including human were M6 and M12. All metabolites detected in rat plasma were < 2% of the parent by AUC. No unique metabolites were detected in human in vitro systems. In human liver microsomes, MVA at outside the range of therapeutic concentrations inhibited CYPs 2C9 and 2C19; also, induced CYP2B6 and CYP3A4, CYP2C8, CYP2C9, and CYP2C19. MVA is not a substrate for P-gp, nor is it a substrate for hepatic transporters, OATPs, OCTs, and NTCP. The potential of MVA to elicit drug-drug interactions via transporters appears low, as in vitro data indicate that MVA at therapeutic concentrations is not an inhibitor of major efflux or uptake transporters. Metabolic oxidation is the primary mechanism of

clearance as evidenced by 58% and 23% of the administered dose that was recovered in the feces and urine, respectively. Small amount was excreted in bile.

Toxicology

General Toxicity studies

The toxic potential of MVA was characterized in single oral dose toxicity study in dogs and repeat oral dose toxicity studies in rats (up to 26 weeks) and dogs (up to 39 weeks). The reversibility of any MVA-related adverse effects was assessed, depending on the duration of the study, over a 4- to 17-week recovery period in repeat dose toxicity studies. Additionally, MVA was evaluated for the potential to cause genotoxicity, reproductive effects, and carcinogenicity risk. The studies were performed with the objective to support chronic oral administration of MVA in patients

Single-dose toxicity study

Oral administration of 1.5, 4.5 and 7 mg MVA/kg in dogs was well tolerated based on the absence of adverse clinical signs. However, single dose of 30 mg/kg resulted in moribund euthanasia at approximately 1 h post dose following clinical signs of extreme hypoactivity, unproductive retching (female), and decreased capillary refill time (male).

Repeat-dose toxicity study

Rats

Oral gavage administration of ≥1.2 mg MVA/kg/day was associated with moribundity and mortality. Clinical observations noted in unscheduled decedents included decreased activity, rapid breathing, piloerection, skin pallor and thinness. The major factor contributing to death/euthanasia was cardiac toxicity resulting in heart failure. Clinical chemistry findings in these animals included increased AST and ALT activities, increased BUN, creatinine and phosphorus concentrations, and decreased total proteins. In animals survived until scheduled necropsy, marked and statistically significant increases in NT-proANP levels relative to control and pretest values were noted at 1/0.6 and 2/1.2 mg/kg/day. Echocardiogram evaluation in surviving animals (≤1.2 mg/kg/day) showed an extensive increase in heart size and a reduction in systolic function as indicated by an increase in LV end diastolic volume and systolic volume, and reduction in LV systolic performance. These effects were reversible by week 10 of the recovery phase in the 26-week study. Histopathologic examination of the heart

showed presence of osseous/cartilaginous metaplasia in males receiving 1.2 mg/kg/day and females receiving ≥0.6 mg/kg/day. This was considered a permanent change and non-reversible. In animals receiving >1.2 mg/kg/day, myocardial hypertrophy (increased heart weights), myocardial degeneration and/or inflammation, endocardial degeneration/necrosis, cardiac hemorrhage, atrial thrombosis, and/or ventricular dilatation were present in the hearts of both unscheduled and scheduled necropsies. Partial recovery was noted for heart findings. Additional microscopic findings included centrilobular necrosis, centrilobular congestion, and/or diffuse hepatocellular vacuolation in the liver, congestion, edema, and/or increased numbers of alveolar macrophages in the lungs; and pancreatic edema were noted in all unscheduled decedents and a few terminal sacrificed rats. The findings were absent in the recovery animals. A non-dose-dependent increase in thyroid/parathyroid weights relative to control was noted in both sexes receiving ≥1 mg MVA/kg/day with no histologic correlates. Based on the histopathological findings, the NOAEL was 0.3 mg/kg/day.

Dogs

The chronic toxicity of MVA was assessed in beagle dogs in an oral gavaging study at doses of 0.06 to 3 mg/kg/day for 6-, 12- and 39-week followed by 4- to 17-week recovery period. At the 3 mg/kg/day, 7/12 dogs died or were euthanized after receiving 6 or 7 doses. The dosing of the group was terminated on day 8. The second high dose, 1 mg/kg/day, was also not tolerated; 8/12 dogs were euthanized after 25/26 doses and, consequently the dosing was terminated. Ante-mortem clinical signs noted at these dose levels included pale gums, prolonged capillary refill time, decreased activity, labored/rapid breathing and pallor. Some animals were noted for body weight loss and decreased food consumption prior to death. As in rats, the major factor contributing to the moribundity/mortality was cardiac toxicity resulting in heart failure. Hematology alterations in some animals included: slight to moderate increase in reticulocyte counts, increase/decrease in red cell mass (considered to reflect regenerative responses secondary to decreased tissue perfusion/oxygenation) and increases in neutrophils and monocytes (considered likely to be secondary to inflammation). Clinical chemistry changes included slightly increased serum AST and ALT activities that correlated with liver congestion seen microscopically, and increases in BUN, creatinine, potassium and phosphorus concentrations that were considered reflective of pre-renal azotemia. Decreased serum chloride concentration noted in one high dose dog was considered to be secondary to gastrointestinal loss associated with emesis. Histopathologic findings were noted in the heart (minimal hemorrhage and inflammatory cell infiltrates and slight myxomatous change and edema in the atrioventricular valve, minimal inflammatory cell infiltrates in the epicardium and minimal degenerative changes in some coronary vessels), lungs (alveolar inflammation and/or increased alveolar macrophages and

minimal lymphocyte apoptosis in the perivascular inflammatory cell infiltrates), lymph nodes and thymus at both high dose levels. Edema and congestion were present in multiple organs and body cavities of unscheduled decedents. Decreased cellularity in the thymus (lower weights and small in size) was noted at all dose levels with partial recovery in animals receiving 1 mg/kg/day.

Studies longer than 6-weeks duration had similar findings with adverse effects on the heart resulting in deaths or euthanization at 0.45 mg/kg/day (12-week study). The euthanized animal demonstrated decreased activity and labored respiration. Cardiac toxicity (severe ventricular dilation) resulting in heart failure was the major factor contributing to the moribundity/mortality. A high dose female terminally euthanized also showed ventricular dilation of the heart. Both dogs had elevated NT-proBNP concentration in their plasma. Echocardiogram evaluations in animals receiving 0.3 (males) or 0.45 mg/kg/day (females) in 39-week study showed substantial increases in mean LV end-diastolic and mean LV end-systolic volumes and a substantial reduction in mean LV ejection fraction. The effects noted on day 76 became progressively more severe on day 256. A complete recovery was noted during the 17-week recovery phase. ECG evaluation showed a marked increase in heart rate associated with prolongation ($P < 0.05$) (11 to 35 msec) of QTc intervals at ≥ 0.18 mg/kg/day relative to predose and control values. Mean QRS duration was prolonged (4 msec) in females receiving 0.4 mg/kg/day. The effects were reversed on day 26 of the recovery phase. The NOAEL was 0.3 mg/kg/day in the 12-week and 0.18 mg/kg/day in the 39-week study for cardiac toxicity and 0.06 mg/kg/day for QTc interval prolongation.

Other Toxicity studies

The impact of impurities (the inactive (R)-enantiomeric impurity MYK-460 at a concentration of (b) (4)% and (b) (4) at a concentration of (b) (4)%) on the toxicological profile of MVA was evaluated in a 3-month oral toxicity study in rats. There was no change in the toxicological profile of MYK-461 as a result of spiking the MVA with both impurities.

Toxicokinetics

Dose linearity and dose proportionality to systemic exposures were investigated in rats and dogs after single and repeated oral dose administration in toxicology studies. Plasma exposure to MVA increase was less than the proportionate dose increment. Accumulation of test substance (an average increase of up to 2.8-fold in rats and up to 9-fold in dogs in Cmax and AUC values) was noted with repeat dosing. Sex-dependent

differences were not noted for either rats or dogs. Plasma levels of MYK-460, the inactive enantiomer of mavacamten and an impurity in the API, were determined as a measure of the extent of in vivo conversion in rats, dogs, and rabbits dosed orally with mavacamten. These data indicate that no unexpected accumulation of MYK-460 was observed relative to mavacamten across species tested and suggest little or no conversion of MYK-461 to MYK-460 in vivo.

Genotoxicity

A standard battery of genetic toxicity tests was performed. MVA was not mutagenic or clastogenic in a battery of in vitro (the Ames reverse mutation assay, micronucleus assay) and *in vivo* (the rat micronucleus assay) studies. Additionally, enantiomer MYK-460 was investigated in vitro in microbial mutagenesis assay (Salmonella typhimurium tester strains), and in an in vitro cytogenetics assay using human lymphocyte cultures. These tests failed to implicate MYK-460 as possessing mutagenic or clastogenic activity. Based on these investigations, it is concluded that both MYK-461 and MYK-460 are a low genotoxic risk to human subjects.

Carcinogenicity

The carcinogenic potential of MVA was evaluated at oral gavage doses of up to 3 mg/kg/day in RasH2 transgenic mice for 26 weeks (completed) and up to 0.6 mg/kg/day in rats for 104 weeks (on-going).

Mouse

Dose selection for the mouse carcinogenicity was based on 1- and 4-week dose range-finding toxicity studies in Wild Type RasH2 male and female mice in which 2 to 8 mg/kg/day of MYK-461 was evaluated (see sections 6.2.1 and 6.2.2).

In the dose range-finding studies, decreased survival was noted at ≥6 mg/kg/day. Increased heart weight, cardiac dilation and cardiac hypertrophy were diagnosed in early decedents. The cause of death was heart failure with dilation of the heart, which correlated with microscopic findings in the left atrium consisting of degeneration/ necrosis, inflammation, thrombus, hemorrhage. The target organs of toxicity were the heart, the liver, kidneys, and lungs with correlative microscopic findings at ≥6 mg/kg/day in both early decedents and those surviving to terminal euthanasia. There were no deaths/euthanasia or histopathologic findings at 5 mg/kg/day (MTD). A decrease ($P < 0.05$) in body weight gain relative to control was noted for males at ≥2 mg/kg/day for

days 1 through 28. A dose-dependent increase (P <0.05) in mean and relative heart weights (attributed to myocardial hypertrophy) was noted at ≥4 mg/kg/day. The NOAEL was 2 mg/kg/day. The NOAEL and mortality dose exposures (Cmax) were 1945 and 5505 ng/ml at 2 and 6 mg/kg/day, respectively. This is 2 to 5 times that achieved in humans (approximately 1000 ng/ml) at the maximum clinical dose of 15 mg/day. Based on these results, the sponsor has proposed dose levels of 1, 2.5 and 5.0 mg/kg/day for the 6-month carcinogenicity in transgenic RasH2 mouse. The Executive CAC did not concur with doses proposed by the sponsor. The Committee recommended doses of 0 (vehicle), 0.5, 1, and 2 (males)/ 3 (females) mg/kg/day based on mortality and significantly reduced body weight gains in 4-week study. The mid- and low- doses were selected based on approximately one third AUC spacing (*see* Appendix D).

In the 26-week CByB6F1- RasH2 transgenic mouse study, oral gavage administration of 0.5, 1, or 2 (males) /3 (females) mg/kg/day MVA did not elicit clinical signs of toxicity except in those that died or were moribund sacrificed. These included hunched appearance, low carriage, irregular respiration, pale appearance, piloerection, limited use of limb, mass (axilla), thin, and swollen vagina. No palpable masses were observed in drug-treated animals. Excluding N-methyl-N-nitrosourea (MNU, positive control) group, there were 8 early decedents including 2 control females during the treatment period. The survival analyses did not show any statistically significant dose response relationship in mortality for both male and female mice. The respective survival rates in the vehicle control, LD, MD, HD, and MNU groups at termination were 100%, 96%, 92%, 100%, and 10% for male mice and 92%, 96%, 96%, 96%, and 20% for female mice. The mortality was statistically significantly higher (p<0.0001) in MNU group compared with vehicle control group. No decrease in body weight or weight changes relative to control was noted at all doses for both males and females. No macroscopic findings were noted in animals examined at unscheduled necropsies or the terminal euthanasia. All macroscopic findings were considered spontaneous and/or incidental because they occurred at a low incidence, were randomly distributed across MYK-461 and control groups. The main target organ for non-neoplastic lesions noted at the high dose group was multiple skeletal muscles. The incidences and types of primary neoplasms that occurred in controls and animals administered MVA were restricted to a few tissues/organs such as thymus, uterus, prostate, lung and blood vessels from multiple tissues. For each tumor type, the sponsor performed statistical analysis if the incidence in at least one test-article treated group was increased by at least two occurrences over the control group for increasing incidence with dose. Based on statistical analyses for neoplastic lesions in MVA treated groups, the sponsor concluded that for both males and females, there were no statistically significant differences in tumor incidence relative to control. The FDA/CDER analysis also showed no evidence of MVA -related tumorigenicity for male or female mice. Also, no statistically significant

increase in incidence rate in test article treated groups when compared with the vehicle control group. Positive control mice treated with MNU were characterized by a high incidence of tumors (malignant lymphoma in multiple locations, especially in the thymus, spleen, and/or other organs; squamous cell papilloma and/or carcinoma in the non-glandular stomach, oral mucosa, and/or skin/subcutis; and choriocarcinoma of the ovary correlating with ovary mass), suggesting a sensitive animal model to identify potential carcinogens. The Executive Carcinogenicity Assessment Committee concurred that the study was adequate and there were no drug-related neoplasms in either males or females (*see* Appendix E).

Rat

MYK-461 is currently being evaluated in a 104-week rat carcinogenicity study. The sponsor will be submitting the results of the study after the approval of the NDA.

Reproductive and Development Toxicity

MYK-461 has been evaluated in fertility, developmental toxicity, and transgenerational reproductive toxicity studies. In segment I fertility and early embryonic development to implantation study, oral administration of MVA at doses up to 1.2 mg/kg/day in male and female rats once during the premating, mating and post-mating or gestation period was well tolerated. The highest dose did not induce early embryonic development toxicity as evidenced by lack of effect on mean number of corpora lutea, implantations, and pre- and post-implantation losses. The NOAEL for this study was 1.2 mg/kg/day, the highest dose tested.

In embryo-fetal development study (formerly known as segment II development toxicity study), oral administration of MVA at doses up to 1.5 mg/kg/day in rats did not cause maternal death or decrease in body weight gain and food consumption. However, the high dose (Cmax 1080 ng/ml; $AUC_{0\text{-}24h}$ 16500 ng.h/ml on gestation day 12) resulted in developmental toxicity as evidenced by increased post-implantation loss, altered fetal growth (such as lower fetal body weight and reduced fetal ossification of bones (cervical and thoracic vertebrae and paws (phalanxes and metatarsals)), visceral (heart) malformations (consisted of total situs inversus (thoracic and abdominal) in one fetus and absence of the right atrioventricular valve associated with a ventricular septum defect in the second fetus) and skeletal malformations (fused sternebrae and absence of lumbar vertebrae). **MVA is teratogenic** with a NOAEL of 0.75 mg/kg/day (Cmax 356 ng/ml; $AUC_{0\text{-}24h}$ 5690 ng.h/ml on gestation day 12) for developmental toxicity. The NOAEL for maternal toxicity was 1.5 mg/kg/day.

In rabbit embryo-fetal development study, MVA at the high dose of 2.0 mg/kg/day caused 2 maternal deaths accompanied by marked body weight loss and little food intake. Bilateral ventricular dilation without any associated microscopic evidence suggests heart failure as the probable cause of death. Surviving animals at ≥1.2 mg/kg/day (Cmax 1100 ng/ml; $AUC_{0\text{-}24h}$ 16500 ng.h/ml on gestation day 12) revealed a mild body weight loss or marked decrease in body weight gain along with a decreased food intake during the dosing period. While there was no effect on pregnancy parameters, the mid and high doses resulted in developmental toxicities as evidenced by test substance-related external (cleft palate), visceral (great vessels) and skeletal (fused sternebrae) malformations and incomplete ossification of a few bones, all suggestive of a **teratogenic potential of MVA**. The NOAEL for maternal and developmental toxicity was 0.6 mg/kg/day (Cmax 516 ng/ml; $AUC_{0\text{-}24h}$ 7160 ng.h/ml on gestation day 12). Also, MVA was quantified in embryonic and extra-embryonic (fetal envelopes, placenta, amniotic fluid) tissues in pregnant animals, the day following the last administration (i.e., 24 h after dosing on GD 12). MVA was distributed in theses tissues, with individual embryonic tissue to plasma concentration ratios ranged between 0.09 and 0.15 in proportion with the dose administered.

In segment III pre- and post-natal development study in rats, MVA at doses up to 1.5 mg/kg/day did not cause F0 maternal systemic toxicity, F1 neonatal/developmental toxicity, F1 parental systemic toxicity, F1 reproductive toxicity, and F2 embryonic toxicity. Based on the lack of adverse effects of MVA, the highest dose (1.5 mg/kg/day) tested was considered the NOAEL for F0 maternal systemic toxicity, F1 neonatal/developmental toxicity, F1 parental systemic toxicity, F1 reproductive toxicity, and F2 embryonic toxicity.

It is concluded that MVA has a high probability of being a **teratogen** when administered during gestation to humans.

Evaluation

Hypertrophic cardiomyopathy (HCM) is an autosomal dominant genetic disease, which is defined clinically as unexplained left ventricle (LV) hypertrophy (Gersh et al. 2011). It is diagnosed as diastolic dysfunction developed as a result of hypercontractility accompanied by reduced ventricular chamber size. In HCM patients, a reduction in cardiac contractile force might decrease LV outflow tract obstruction by counteracting the excessive thickening of the left ventricular wall of the outflow tract. A reduction in sarcomere contraction resulting 10 to 15% decrease in left ventricular ejection fraction relative to baseline might be beneficial in HCM patients. There are no sarcomere-

targeted therapies to attenuate the hypercontractility associated with HCM. In this context, the new drug mavacamten (MYK-461), a small molecular weight, allosteric, and reversible inhibitor of cardiac myosin inhibitor has been demonstrated to relieve hypercontraction and LVOT obstruction in HCM patients. It does so by selectively targeting cardiac myosin and limiting the number of myosin heads that can enter on actin (power-generating) states. This action reduces force-producing (systolic) and residual (diastolic) cross-bridge formation resulting in the reduction of sarcomere contractility facilitating diastolic relaxation, improving both dynamic LVOT obstruction and LV compliance in patients with HCM, thus targeting the pathophysiology of the disease.

Cardiac performance evaluated in rodents and dogs showed exposure-dependent inhibition of fractional shortening (~5% to 80%) while markedly increasing EDV and reducing dP/dtmax after single oral doses of 0.045 to 8 mg MVA/kg. A plot between measured plasma concentrations and FS showed a non-linear dose-dependent FS with no plateau in effect (Fig 22B, 44A), posing the risk of fast deterioration of cardiac contractility and achieving exposures that are not well tolerated. Dogs could not tolerate a single i.v. dose over 1.2 mg/kg and a single oral dose over 7 mg/kg. In repeat dose toxicity studies, deaths or euthanization occurred in rats at 1.2 and in dogs at 0.45 mg/kg/day (Table 145). Severe reduction in cardiac contractility accompanied by a decrease in systolic blood pressure and an increase in heart rate contributed to cardiac toxicity culminating in heart failure. This is seen as excessive on-target toxicity (heart failure) in animals with normal contractility at slightly higher doses, suggesting a possible narrow margin of safety as a mere < 3- (dogs) to 6- (rats) fold increase in dose results in mortality (Table 145). Thus, a modest reduction (~15-20%) in contractility is thought to be sufficiently beneficial in HCM patients that would allow LV remodeling back to normal without eliciting excessive pharmacologic effects.

Beta-adrenergic agonists, isoproterenol and dobutamine, oppose the effect by restoring the contractility to 60% of baseline. However, the reversal is transient given the long-lasting effect of MVA on the heart. Thus, other measures should be in place while ascending oral doses of MVA are considered. Unlike traditional negative inotropes, MVA can induce direct and sustained negative inotropy (normalizing systolic function in HCM) while simultaneously improving diastolic cardiac filling and stresses. MVA induced QT and QTc interval prolongation in both acute (delayed effect) and chronic studies in both rats and dogs. Mechanistically it could be the result of multi-ion channel blockade as there was no effect on the hERG ion channel, action potential duration and early afterdepolarizations or as adaptive response to sustained myosin inhibition in ventricles with normal physiology. The totality of data suggests that MVA has a low torsadogenic or proarrhythmic risk. **The NOAEL for QTc interval prolongation in**

dogs is 0.06 mg/kg/day, which is 0.08 to 0.1 times human exposure (AUC) at the MRHD (Table 146).

No off-target toxicity was observed in animal studies of mavacamten, and adverse toxicity was related to excessive pharmacologic effects on the heart. Also, acute mavacamten administration did not have any noteworthy cardiorespiratory or CNS effects at doses up to 10 mg/kg.

Dog is more sensitive than is the rat having demonstrated a NOAEL of 0.18 and 0.3 mg/kg/day, respectively (Table 145). In dogs, the drug is highly bioavailable with large volume of distribution especially left ventricle to plasma ratio > 20 and long half-life (~7 days). Repeated dosing resulted in accumulation of MVA (up to 2.8-fold in rats and 9-fold in dogs). Cardiac toxicity (inhibition of contractility) resulting in HF was noted at plasma concentrations of 725 ng/ml (a dose of 1.2 mg/kg/day in 26-week toxicity study) in rats and 911 ng/ml in dogs (a dose of 0.45 mg/kg/day in 13-week toxicity study) (Table 145). Other target organs of toxicity in both rats and dogs are liver (centrilobular necrosis, congestion and vacuolation), lungs (increased alveolar macrophages and inflammation) and thymus (decrease in size with decreased cellularity). At supratherapeutic exposures, increases in BUN, creatinine, potassium, and phosphorous concentrations that are considered reflective of pre-renal azotemia were noted in the rat and dog. Furthermore, edema and congestion were present in multiple organs and body cavities. All these findings were reversible except for partial reversibility in the heart histopathology.

Mavacamten was not mutagenic or genotoxic in vivo and in vitro assays. No evidence of carcinogenic potential has been identified in the 6-month transgenic mouse study, and results from the 24-month rat study are pending. Mavacamten did not cause transgenerational reproductive effects or adversely affect fertility of male or female rats when repeatedly dosed. However, in embryo-fetal development study in rats, mavacamten at 1.5 mg/kg/day increased post-implantation loss, lowered mean fetal body weight, slightly reduced fetal skeletal ossification, induced heart malformation (total situs inversus), and increased incidences of skeletal malformations relative to control. In rabbits, increased incidences of cleft palate, great vessel malformations (dilatation of pulmonary trunk and/or aortic arch), and fused sternebrae in fetuses were observed at the same doses that caused maternal toxicity. Mavacamten has a high probability of being **a teratogen** when administered during gestation as evidenced in both the rat and rabbit embryo-fetal development studies. The NOAEL for MVA in these studies was 0.75 mg/kg/day (C_{max} 356 ng/ml; $AUC_{0\text{-}24h}$ 5690 ng.h/ml on gestation day 12) in rats, and 0.6 mg/kg/day (C_{max} 516 ng/ml; $AUC_{0\text{-}24h}$ 7160 ng.h/ml on gestation day 12) in rabbits (Table 146). Exposure of the fetus, placenta, and amniotic fluid to MVA was demonstrated in pregnant rabbits (on GD 12). MVA was distributed in theses

tissues, with individual embryonic tissue to plasma concentration ratios ranging from 0.09 to 0.15 across the dose levels. It is not known whether mavacamten excretes into the milk.

Table 145. Comparison of dose or exposure (Cmax) of MYK-461 in rats and dogs at NOAEL and mortality doses

Species	Toxicology				Ratio of Death to NOAEL	
	NOAEL		Death			
	Dose	Cmax	Dose	Cmax	Based on the dose	Based on the exposure
Rats[a]	0.3	120	1.2	725	4.0	6.0
Dogs[b]	0.18	388 M 288 F	0.45	1040 M 782 F	2.5	M 2.7 F 2.7

Dose is expressed as mg/kg/day, Cmax is expressed as ng/ml

a: data from 26-week study, Cmax on day 182, males and females combined
b: data from 3-month study, Cmax for NOAEL on Day 86 for males and females; Cmax for mortality, on day 73 for males and day 86 for females.

Plasma exposure in all toxicology studies and reproductive toxicology studies at the NOAEL is less than those in humans at the MRHD (Table 146) indicating the observed toxicities could be relevant at the therapeutic dose range. In pharmacology studies, MVA is slightly more potent and efficacious in the rat and dog, making them in toxicologic assessment more sensitive to the cardiac toxicities than in human. It is also recognized that the indicated patient population has hypercontractile myocardium and would likely tolerate the reduction in cardiac contractility to a moderate extent. The only exception for this low margin of safety is the Tg mouse 26-week carcinogenicity study in which there were no findings suggesting mavacamten was carcinogenic at the highest doses tested. The safety margins for male and female mice for carcinogenic potential were 1.8 to 2.3 and 3.0 to 4.4, respectively, times the exposure in humans at the MRHD (Table 146). The results from a 104-week rat carcinogenicity study are pending.

Table 146. Serious dose-related adverse effects observed in toxicology studies with mavacamten. Animal to human exposure ratios is calculated based on MRHD of 15 mg/day.

Species GLP Tox study	Dose, mg/kg /day	Effects		PK data-Tox study		Multiple, Animal/human[1]	
		Significant findings	Parame-ter	Cmax ng/ml	AUC ng.hr/ml	Cmax	AUC
Rat, 26-wk[2]	**0.3**		**NOAEL**	120	1670	0.12	0.1
	1.2	Deaths from HF, extensive increase in heart size and a reduction in systolic function	Death	725	10700	0.80	0.63
Dog 13-/39-week[3]	**0.06**	Absence of QTc prolongation	**NOAEL**	120	1580	0.12	0.09
	0.18	QTc interval prolongation[4] NOAEL is 0.06 mg/kg/day	**NOAEL**	M 388 F 288	M 6220 F 3800	0.40 0.30	0.37 0.22
	0.45	Cardiac toxicity (severe ventricular dilation) resulting in heart failure. QTc interval prolongation[4]	Death	M 1040[5] F 782[6]	M 17200 F 12300	1.10 0.81	1.02 0.73
Carcinogenicity							
RasH2 mouse[7]	M 2 F 3	No tumorigenic findings	**NOAEL**	M 2230 F 4280	M 30000 F 50400	2.30 4.45	1.78 3.00
Fertility							
Rat	1.2	Fertility and early embryonic development to implantation	**NOAEL**	NA	NA	-	-
Embryo-fetal Development Toxicity							
Rat[10]	1.5	Teratogenic potential[11]		1080	16500	1.12	0.98
	0.75	Developmental	**NOAEL**	356	5690	0.40	0.34
	1.5	Maternal	**NOAEL**	1080	16500	1.12	0.98
Rabbit[10]	1.2	Teratogenic potential		1100	16500	1.14	0.98
	0.6	Maternal and developmental	**NOAEL**	516	7160	0.54	0.42
Pre- and Postnatal Development Toxicity							
Rat[12]	1.5	F_0 Maternal, F1 developmental and reproductive, F_2 embryonic	**NOAEL**	1080	16500	1.12	0.98

Male and female combined Cmax and AUC0-24 values unless specified. M: male; F: female
1: Based on EXPLORER-HCM study at a dose of 15 mg MYK-461/day for 10 days. Mean Cmax: 962 ng/ml, AUC_{0-24} 16,891 h*ng/mL used for calculating exposure multiples (Sponsor email of 6/14/2021).
2: PK data measured on day 182
3: PK data from 13-week study, measured on day 73 for males at 0.45 mg/kg/day and the rest on day 86.
4: 39-week study: ECG evaluation showed prolongation ($P < 0.05$) of QTc intervals in animals receiving ≥ 0.18 mg/kg/day relative to predose and control values. Echocardiogram evaluations indicated substantial increases in mean LV end-diastolic and a substantial reduction in mean LV ejection fraction.
5: Two males died/euthanized on days 70, 72
6: One female euthanized on day 93
7: TK measurements on day 182
10: Exposure data in reproductive toxicity were based on gestation day 12
11: At 1.5 mg/kg/day, increased post-implantation loss, altered fetal growth (such as lower fetal body weight and reduced fetal ossification of bones (cervical and thoracic vertebrae and), visceral (heart) malformations and skeletal malformations were observed.
12: Plasma concentrations of MYK-461 were not assessed in this study. Exposure multiples were based on the PK data in Embryo-fetal development study (see foot note #10).

Conclusions

Mavacamten is an allosteric and reversible inhibitor of cardiac myosin developed for the treatment of oHCM that directly targets the pathophysiology of the disease. MVA was evaluated for its mechanism of action and its novel pharmacology, proarrhythmogenic liabilities, and for the potential to cause tissue injury, genotoxicity, reproductive effects, and carcinogenicity risk. The consistent findings in all toxicity studies have been adverse effects in the heart (decreased cardiac contractility resulting in unintended deaths or cardiac failure) consistent with the pharmacologic effects of MVA. MVA exhibited no off-target toxicity and is considered to have a low torsadogenic or proarrhythmic risk although QT interval prolongation was noted in animal studies concomitant with sustained myosin inhibition. Mavacamten was not genotoxic. It did not affect fertility of male or female rats, nor did it affect the fertility of F1 offspring of female rats dosed with MVA during gestation. However, the drug was found to be teratogenic in rats and rabbits at a very low dose. In all toxicity studies (except carcinogenicity), plasma exposure (Cmax and AUC) at the no-observed adverse effect level is less than those in humans at the MRHD suggesting potential risk even at the therapeutic dose range. Finally, there are no non-clinical findings that preclude from the approvability of MVA to patients with symptomatic HCM to reduce LVOT obstruction and ventricular filling pressures. Also, the data suggest that MVA can be used safely in these patients group at the recommended therapeutic dose and in accordance with the proposed product labeling.

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13 Appendix/Attachments

Appendix A: Sponsor's data: 6-month carcinogenicity study in TG- rasH2 mice. Summary of group mean body weights.

Appendix B: Sponsor's data: 6-month carcinogenicity study in TG- rasH2 mice. Summary incidence of tumor findings.

Appendix C: Sponsor's data: 6-month carcinogenicity study in TG- rasH2 mice. Incidence of non-neoplastic findings.

Appendix D: CAR Protocol assessment. Executive CAC Meeting Minutes – June 12, 2018.

Appendix E: Final CAR study minutes. Executive CAC Meeting – July 20, 2021.

NDA 214998. Supplementary Table A. 6-month carcinogenicity study in TG- rasH2 mice.
Summary of group mean body weights (g)

Day	Male (mg/kg/day)				Female (mg/kg/day)			
	Control	0.5	1	2	Control	0.5	1	3
1	25.6	25.6	25.6	25.6	20.9	20.9	21.1	21.0
8	24.8	24.9	24.9	25.1	20.2	20.5	20.6	20.4
15	24.9	25.2	25.0	25.0	20.2	20.5	20.6	20.5
22	24.9	25.3	25.2	25.4	20.5	20.5	20.6	20.6
29	25.1	25.5	25.6	25.4	20.9	20.8	21.1	20.6
36	25.5	25.8	25.7	25.6	20.9	20.8	20.8	20.6
43	25.6	25.9	25.8	26.0	20.9	20.9	21.5	21.2
50	25.6	25.9	25.9	26.1	21.4	21.3	21.1	21.2
57	25.6	26.0	25.7	26.1	21.3	21.2	21.5	21.2
64	25.9	26.1	26.1	26.4	21.5	21.5	21.7	21.2
71	26.2	26.6	26.7	26.9	21.9	21.8	21.6	21.4
78	26.2	26.5	26.6	26.8	21.9	22.2	22.0	21.8
85	26.5	27.1	26.9	27.2	21.9	22.0	22.3	22.0
92	26.4	27.0	27.1	27.3	22.4	22.5	22.6	22.0
99	26.5	27.1	27.2	27.6	22.2	22.7	22.2	22.0
106	26.8	27.2	27.7	27.8	22.3	22.4	22.2	21.7
113	27.0	27.6	27.6	28.0	22.7	22.8	22.5	21.9
120	27.2	28.0	27.8	28.2	22.6	22.2	22.6	21.5
127	27.1	27.8	27.7	28.3	22.5	22.4	22.3	22.0
134	27.6	28.2	27.8	28.2	22.8	22.5	22.7	22.2
141	27.8	28.5	28.2	28.4	23.2	22.6	23.0	22.3
148	27.8	28.4	28.3	28.7	22.8	22.8	23.0	22.2
155	27.7	28.8	29.0	28.6	22.7	23.2	23.4	22.9
162	28.0	29.1	28.5	28.9	23.1	23.7	23.9	23.0
169	27.9	28.8	28.5	29.1	23.3	23.4	23.5	22.9
176	27.7	28.5	28.1	28.6	23.2	23.7	23.4	22.7
182	27.7	28.6	28.3	28.7	23.5	23.5	23.5	23.2

NDA 214998. Supplementary Table B. 6-month carcinogenicity study in TG- rasH2 mice. Summary incidence of tumor findings

Organ/Tissue	Finding	Malignancy Status	Male				Female			
			Control	0.5	1	2	Control	0.5	1	3
skin/subcutis	carcinoma, squamous cell, malignant	M								
	hemangiosarcoma, malignant						1[a]			
	hemolymphoreticular tumor, malignant	MT					1			
	papilloma, squamous cell, benign	B								
hemolymphoreticular tissue	lymphoma, malignant	M		1			1	1		
stomach, nonglandular	carcinoma, squamous cell, malignant									
	hemangiosarcoma, malignant				1					
	hemolymphoreticular tumor, malignant	MT					1			
	papilloma, squamous cell, benign	B				1				
thymus	hemangiosarcoma, malignant	M						1		
	hemolymphoreticular tumor, malignant	MT					1			
	thymoma, benign	B					3	1	1	1
	thymoma, malignant	M		1				1		
spleen	hemangioma, benign	B								1
	hemangiosarcoma, malignant	M	1	1	3	1	1	2	1	2
	hemolymphoreticular tumor, malignant	MT		1						
lung	adenoma, bronchioloalveolar, benign	B	4	2	2	4			1	1
	carcinoma, bronchioloalveolar, malignant	M			2			1	1	
	hemolymphoreticular tumor, malignant	MT		1			1			
stomach, glandular	carcinoma, squamous cell, malignant									
	hemolymphoreticular tumor, malignant									
uterus	hemangiosarcoma, malignant	M					3	3	1	2
	hemolymphoreticular tumor, malignant	MT					1			
	polyp, endometrial stromal, benign	B						1	1	
liver	hemolymphoreticular tumor, malignant	MT		1			1			
gland, harderian	adenocarcinoma, malignant	M		1				1		

Organ/Tissue	Finding	Malignancy Status	Male				Female			
			Control	0.5	1	2	Control	0.5	1	3
	adenoma, benign	B		1				1		
kidney	hemolymphoreticular tumor, malignant	MT								
lymph node, mandibular										
lymph node, mesenteric										
ovary	choriocarcinoma, malignant	M								
	hemolymphoreticular tumor, malignant	MT					1			
bone marrow, femur				1						
bone marrow, sternum	hemangiosarcoma, malignant	M	1							
	hemolymphoreticular tumor, malignant	MT		1						
bone, sternum	hemangiosarcoma, malignant		1							
brain	hemolymphoreticular tumor, malignant							1		
cervix	hemangiosarcoma, malignant							1		
	hemolymphoreticular tumor, malignant						1			
eye							1			
	melanoma, benign	B							1	
gallbladder	hemolymphoreticular tumor, malignant	MT					1			
gland, clitoral	hemangiosarcoma, malignant							1		
	hemolymphoreticular tumor, malignant						1			
gland, lacrimal							1			
gland, prostate	sarcoma, malignant	M			1					
gland, salivary, parotid	hemolymphoreticular tumor, malignant	MT					1			
gland, salivary, submandibular							1			
heart							1			
large intestine, rectum	hemangiosarcoma, malignant	M				1			1	
		MT			1					
lymph node	hemolymphoreticular tumor, malignant									
mucosa, oral	papilloma, squamous cell, benign	B								
nasal turbinate	hemolymphoreticular tumor, malignant	MT		1						

Organ/Tissue	Finding	Malignancy Status	Male				Female			
			Control	0.5	1	2	Control	0.5	1	3
oviduct							1			
small intestine, duodenum										
testis	hemangiosarcoma, malignant	MA	1							
urinary bladder	hemolymphoreticular tumor, malignant	MT					1			
vagina	hemangiosarcoma, malignant	MT						1		
	hemolymphoreticular tumor, malignant	MT					1			

B: Benign, MA: Malignant, MT: Metastatic. a: number of occurrences 2

NDA 214998. Supplementary Table C. 6-month carcinogenicity study in TG- rasH2 mice. Incidence of non-neoplastic findings

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
vagina	Cyst	multifocal								1 (4.0%)
										1 (4.0%)
	Hyperplasia, epithelium	focal								
uterus	Hyperplasia, angiomatous	focal								1 (4.0%)
										1 (4.0%)
	Hyperplasia, cystic endometrial	focal; multifocal						3 (12.0%)	1 (4.0%)	
		multifocal					2 (8.0%)			1 (4.0%)
							2 (8.0%)	3 (12.0%)	1 (4.0%)	1 (4.0%)
urinary bladder	Infiltrate, mononuclear cell	focal; multifocal	6 (24.0%)	7 (28.0%)	6 (24.0%)	8 (32.0%)	10 (40.0%)	17 (68.0%)	13 (56.5%)	11 (45.8%)
		multifocal								1 (4.2%)
			6 (24.0%)	7 (28.0%)	6 (24.0%)	8 (32.0%)	10 (40.0%)	17 (68.0%)	13 (56.5%)	12 (50.0%)
	Inflammation, neutrophilic	multifocal			1 (4.0%)					
					1 (4.0%)					
ureter	Inflammation, mixed cell	bilateral			1 (4.0%)					
					1 (4.0%)					
tongue	Inflammation, vessel	multifocal							1 (4.0%)	
									1 (4.0%)	
thymus	Cellularity, decreased, lymphocyte	focal; multifocal							2 (8.3%)	
		diffuse; multifocal					2 (8.0%)	1 (4.0%)		1 (4.2%)
		diffuse			1 (4.0%)					
					1 (4.0%)		2 (8.0%)	1 (4.0%)	2 (8.3%)	1 (4.2%)
	Cellularity, increased, epithelial cell	focal; multifocal							3 (12.5%)	
		multifocal						1 (4.0%)		2 (8.3%)
								1 (4.0%)	3 (12.5%)	2 (8.3%)
	Cyst					1 (4.0%)	2 (8.0%)	1 (4.0%)	3 (12.5%)	1 (4.2%)
						1 (4.0%)	2 (8.0%)	1 (4.0%)	3 (12.5%)	1 (4.2%)
testis	Degeneration/atrophy, tubular	bilateral; unilateral	2 (8.0%)	3 (12.0%)	1 (4.0%)	1 (4.0%)				
		unilateral		2 (8.0%)						

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
		bilateral				1 (4.0%)				
						1 (4.0%)				
			2 (8.0%)	5 (20.0%)	1 (4.0%)	3 (12.0%)				
	Hyperplasia, Leydig cell	diffuse				1 (4.0%)				
						1 (4.0%)				
	Infiltrate, mononuclear cell	unilateral		1 (4.0%)						
				1 (4.0%)						
stomach, nonglandular	Hyperplasia, squamous cell	focal	1 (4.0%)							
			1 (4.0%)							
	Infiltrate, mononuclear cell	focal; multifocal	1 (4.0%)	2 (8.0%)	1 (4.0%)					
			1 (4.0%)	2 (8.0%)	1 (4.0%)					
	Ulcer	focal; pylorus								1 (4.0%)
		focal								
										1 (4.0%)
stomach, glandular	Hyperplasia, epithelium	focal								
	Infiltrate, mixed cell	focal; multifocal	2 (8.0%)	3 (12.0%)	4 (16.0%)	1 (4.0%)	1 (4.0%)		3 (12.0%)	1 (4.0%)
		focal		1 (4.0%)						
			2 (8.0%)	4 (16.0%)	4 (16.0%)	1 (4.0%)	1 (4.0%)		3 (12.0%)	1 (4.0%)
spleen	Hematopoiesis, extramedullary, increased	diffuse							1 (4.0%)	
							1 (4.0%)			
							1 (4.0%)		1 (4.0%)	
	Pigment, melanin	focal; multifocal	1 (4.0%)	3 (12.0%)	1 (4.0%)		4 (16.0%)	1 (4.0%)	3 (12.0%)	3 (12.0%)
			4 (16.0%)	4 (16.0%)	4 (16.0%)	2 (8.0%)	4 (16.0%)	2 (8.0%)	3 (12.0%)	
			5 (20.0%)	7 (28.0%)	5 (20.0%)	2 (8.0%)	8 (32.0%)	3 (12.0%)	6 (24.0%)	3 (12.0%)
spinal cord	Cyst, squamous					1 (4.0%)				
						1 (4.0%)				
	Diverticulum	multifocal		1 (4.0%)						

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
small intestine, duodenum				1 (4.0%)						
	Hyperplasia, epithelium	focal				1 (4.0%)				
						1 (4.0%)				
skin/subcutis	Hemorrhage	diffuse; subcutis					1 (4.0%)			
							1 (4.0%)			
	Infiltrate, mixed cell	multifocal					1 (4.0%)		1 (4.0%)	1 (4.0%)
		diffuse; subcutis; multifocal					1 (4.0%)	1 (4.0%)		
							2 (8.0%)	1 (4.0%)	1 (4.0%)	1 (4.0%)
	Infiltrate, mononuclear cell	focal; multifocal		2 (8.0%)	2 (8.0%)					1 (4.0%)
				2 (8.0%)	2 (8.0%)					1 (4.0%)
pancreas	Ectasia, duct	focal							1 (4.0%)	
									1 (4.0%)	
	Infiltrate, mononuclear cell	focal; multifocal		2 (8.0%)	1 (4.0%)				1 (4.0%)	1 (4.0%)
		focal					1 (4.0%)			
				2 (8.0%)	1 (4.0%)		1 (4.0%)		1 (4.0%)	1 (4.0%)
	Inflammation, mixed cell	focal								1 (4.0%)
		focal; periductal							1 (4.0%)	
									1 (4.0%)	1 (4.0%)
ovary	Corpus, hemorrhagicum	focal						1 (4.2%)		
								1 (4.2%)		
nasal turbinate	Cyst, squamous	focal	1 (4.0%)					1 (4.0%)		
			1 (4.0%)					1 (4.0%)		
	Eosinophilic globules	multifocal; multifocal; respiratory epithelium	3 (12.0%)	5 (20.0%)	1 (4.0%)		3 (12.0%)		1 (4.2%)	3 (12.0%)
		multifocal						1 (4.0%)	1 (4.2%)	
		multifocal; multifocal; respiratory epithelium				1 (4.2%)		1 (4.0%)		1 (4.0%)
			3 (12.0%)	5 (20.0%)	1 (4.0%)	1 (4.2%)	3 (12.0%)	2 (8.0%)	2 (8.3%)	4 (16.0%)

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
	Hyperplasia, respiratory epithelium	focal; multifocal		1 (4.0%)			1 (4.0%)	1 (4.0%)		
		multifocal						1 (4.0%)		
		focal; multifocal				1 (4.2%)				1 (4.0%)
				1 (4.0%)		1 (4.2%)	1 (4.0%)	2 (8.0%)		1 (4.0%)
	Inflammation, mixed cell	focal; multifocal	4 (16.0%)	7 (28.0%)	8 (32.0%)	4 (16.7%)	6 (24.0%)	8 (32.0%)	9 (37.5%)	8 (32.0%)
				1 (4.0%)		1 (4.2%)				
			4 (16.0%)	8 (32.0%)	8 (32.0%)	5 (20.8%)	6 (24.0%)	8 (32.0%)	9 (37.5%)	8 (32.0%)
muscle, soleus - right	Degeneration/regeneration	focal; multifocal	8 (33.3%)	8 (32.0%)	13 (52.0%)	12 (48.0%)	8 (32.0%)	4 (16.0%)	5 (20.8%)	7 (28.0%)
			8 (33.3%)	8 (32.0%)	13 (52.0%)	12 (48.0%)	8 (32.0%)	4 (16.0%)	5 (20.8%)	7 (28.0%)
	Infiltrate, mixed cell	focal; multifocal	2 (8.3%)		2 (8.0%)	1 (4.0%)				
			2 (8.3%)		2 (8.0%)	1 (4.0%)				
	Infiltrate, mononuclear cell	focal; multifocal	1 (4.2%)	3 (12.0%)		2 (8.0%)	1 (4.0%)	2 (8.0%)		
			1 (4.2%)	3 (12.0%)		2 (8.0%)	1 (4.0%)	2 (8.0%)		
muscle, soleus - left	Degeneration/regeneration	focal; multifocal	10 (41.7%)	10 (40.0%)	11 (44.0%)	6 (24.0%)	6 (24.0%)	6 (24.0%)	7 (29.2%)	9 (36.0%)
			10 (41.7%)	10 (40.0%)	11 (44.0%)	6 (24.0%)	6 (24.0%)	6 (24.0%)	7 (29.2%)	9 (36.0%)
	Infiltrate, mixed cell	focal		1 (4.0%)				1 (4.0%)		
				1 (4.0%)				1 (4.0%)		
	Infiltrate, mononuclear cell	focal; multifocal	1 (4.2%)	4 (16.0%)		1 (4.0%)	3 (12.0%)	1 (4.0%)		1 (4.0%)
			1 (4.2%)	4 (16.0%)		1 (4.0%)	3 (12.0%)	1 (4.0%)		1 (4.0%)
muscle, quadriceps femoris	Degeneration/regeneration	multifocal	25 (100.0%)	23 (92.0%)	25 (100.0%)	24 (96.0%)	25 (100.0%)	25 (100.0%)	24 (96.0%)	25 (100.0%)
			25 (100.0%)	23 (92.0%)	25 (100.0%)	24 (96.0%)	25 (100.0%)	25 (100.0%)	24 (96.0%)	25 (100.0%)
	Infiltrate, mixed cell	focal; multifocal		1 (4.0%)			2 (8.0%)	1 (4.0%)		2 (8.0%)
		multifocal		1 (4.0%)			1 (4.0%)			
				2 (8.0%)			3 (12.0%)	1 (4.0%)		2 (8.0%)
	Infiltrate, mononuclear cell	focal; multifocal	12 (48.0%)	11 (44.0%)	15 (60.0%)	13 (52.0%)	17 (68.0%)	14 (56.0%)	16 (64.0%)	11 (44.0%)
			12 (48.0%)	11 (44.0%)	15 (60.0%)	13 (52.0%)	17 (68.0%)	14 (56.0%)	16 (64.0%)	11 (44.0%)
	Inflammation, vessel	focal							1 (4.0%)	
									1 (4.0%)	
muscle, gastrocnemius	Degeneration/regeneration	focal; multifocal	24 (96.0%)	23 (92.0%)	25 (100.0%)	24 (96.0%)	24 (100.0%)	25 (100.0%)	24 (96.0%)	24 (96.0%)
		multifocal	1 (4.0%)							

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
			25 (100.0%)	23 (92.0%)	25 (100.0%)	24 (96.0%)	24 (100.0%)	25 (100.0%)	24 (96.0%)	24 (96.0%)
	Infiltrate, mixed cell	focal; multifocal		3 (12.0%)	2 (8.0%)		4 (16.7%)	1 (4.0%)	3 (12.0%)	
		multifocal		1 (4.0%)						
				4 (16.0%)	2 (8.0%)		4 (16.7%)	1 (4.0%)	3 (12.0%)	
	Infiltrate, mononuclear cell	focal; multifocal	9 (36.0%)	4 (16.0%)	6 (24.0%)	5 (20.0%)	6 (25.0%)	8 (32.0%)	7 (28.0%)	6 (24.0%)
			9 (36.0%)	4 (16.0%)	6 (24.0%)	5 (20.0%)	6 (25.0%)	8 (32.0%)	7 (28.0%)	6 (24.0%)
muscle, diaphragm	Infiltrate, mixed cell	focal				1 (4.0%)				
						1 (4.0%)				
	Infiltrate, mononuclear cell	focal; multifocal					2 (8.0%)		1 (4.2%)	
							2 (8.0%)		1 (4.2%)	
muscle, biceps femoris	Degeneration/regeneration	multifocal	24 (96.0%)	23 (92.0%)	25 (100.0%)	24 (96.0%)	24 (100.0%)	24 (96.0%)	24 (96.0%)	23 (92.0%)
			1 (4.0%)							
			25 (100.0%)	23 (92.0%)	25 (100.0%)	24 (96.0%)	24 (100.0%)	24 (96.0%)	24 (96.0%)	23 (92.0%)
	Infiltrate, mixed cell	focal; multifocal	1 (4.0%)	1 (4.0%)	4 (16.0%)	1 (4.0%)	3 (12.5%)		1 (4.0%)	
		multifocal		1 (4.0%)						
			1 (4.0%)	2 (8.0%)	4 (16.0%)	1 (4.0%)	3 (12.5%)		1 (4.0%)	
	Infiltrate, mononuclear cell	focal; multifocal	6 (24.0%)	7 (28.0%)	7 (28.0%)	8 (32.0%)	8 (33.3%)	7 (28.0%)	4 (16.0%)	4 (16.0%)
			6 (24.0%)	7 (28.0%)	7 (28.0%)	8 (32.0%)	8 (33.3%)	7 (28.0%)	4 (16.0%)	4 (16.0%)
lymph node, mesenteric	Inflammation, vessel	focal		1 (4.0%)						
				1 (4.0%)						
lung	Hemorrhage	multifocal				1 (4.0%)				
			1 (4.0%)							
							1 (4.0%)			
			1 (4.0%)			1 (4.0%)	1 (4.0%)			
	Hyperplasia, bronchiolo-alveolar	multifocal				1 (4.0%)				
		focal			1 (4.0%)					
					1 (4.0%)	1 (4.0%)				
	Infiltrate, macrophages, alveolus	focal; multifocal		1 (4.0%)	1 (4.0%)	2 (8.0%)	1 (4.0%)		1 (4.0%)	
		focal			1 (4.0%)		1 (4.0%)			
				1 (4.0%)	2 (8.0%)	2 (8.0%)	2 (8.0%)		1 (4.0%)	

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
	Inflammation, mixed cell	focal	1 (4.0%)			1 (4.0%)				
			1 (4.0%)		1 (4.0%)	1 (4.0%)				
			2 (8.0%)		1 (4.0%)	2 (8.0%)				
	Mineralization	focal			1 (4.0%)					
					1 (4.0%)					
liver	Congestion	multifocal	1 (4.0%)							
			1 (4.0%)							
	Focus, cellular alteration, basophilic	focal		2 (8.0%)		1 (4.0%)				1 (4.0%)
				2 (8.0%)		1 (4.0%)				1 (4.0%)
	Hematopoiesis, extramedullary	diffuse					1 (4.0%)			
							1 (4.0%)			
	Hemorrhage	focal				1 (4.0%)				
						1 (4.0%)				
	Infiltrate, mixed cell	focal; multifocal	7 (28.0%)	9 (36.0%)	7 (28.0%)	8 (32.0%)	9 (36.0%)	17 (68.0%)	14 (56.0%)	11 (44.0%)
			7 (28.0%)	9 (36.0%)	7 (28.0%)	8 (32.0%)	9 (36.0%)	17 (68.0%)	14 (56.0%)	11 (44.0%)
	Infiltrate, mononuclear cell	focal; multifocal	2 (8.0%)	5 (20.0%)	4 (16.0%)	5 (20.0%)	10 (40.0%)	5 (20.0%)	8 (32.0%)	11 (44.0%)
			2 (8.0%)	5 (20.0%)	4 (16.0%)	5 (20.0%)	10 (40.0%)	5 (20.0%)	8 (32.0%)	11 (44.0%)
	Necrosis, hepatocyte	focal; multifocal	6 (24.0%)	8 (32.0%)	5 (20.0%)	2 (8.0%)		3 (12.0%)	6 (24.0%)	4 (16.0%)
		focal		1 (4.0%)						
		diffuse; lobe; left lateral								
			6 (24.0%)	9 (36.0%)	5 (20.0%)	2 (8.0%)		3 (12.0%)	6 (24.0%)	4 (16.0%)
	Thrombus	focal				1 (4.0%)				
						1 (4.0%)				
larynx	Degeneration, muscularis	focal	2 (8.0%)	2 (8.0%)	1 (4.0%)	2 (8.0%)	3 (12.0%)	1 (4.0%)	1 (4.2%)	1 (4.0%)
			2 (8.0%)	2 (8.0%)	1 (4.0%)	2 (8.0%)	3 (12.0%)	1 (4.0%)	1 (4.2%)	1 (4.0%)
	Infiltrate, mixed cell	focal; multifocal			1 (4.0%)		1 (4.0%)	1 (4.0%)		
					1 (4.0%)		1 (4.0%)	1 (4.0%)		
large intestine, rectum	Necrosis, fat	focal						1 (4.0%)		
								1 (4.0%)		

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
kidney	Basophilic tubule	focal; multifocal	7 (28.0%)	8 (32.0%)	6 (24.0%)	7 (28.0%)	3 (12.0%)	1 (4.0%)	2 (8.0%)	3 (12.0%)
		multifocal			1 (4.0%)	1 (4.0%)				
			7 (28.0%)	8 (32.0%)	7 (28.0%)	8 (32.0%)	3 (12.0%)	1 (4.0%)	2 (8.0%)	3 (12.0%)
	Dilatation, tubule(s)	focal; multifocal						1 (4.0%)		1 (4.0%)
		focal			1 (4.0%)					
					1 (4.0%)			1 (4.0%)		1 (4.0%)
	Infiltrate, fat	focal		1 (4.0%)						
				1 (4.0%)						
	Infiltrate, mononuclear cell	focal; multifocal	24 (96.0%)	23 (92.0%)	23 (92.0%)	22 (88.0%)	22 (88.0%)	25 (100.0%)	23 (92.0%)	20 (80.0%)
			24 (96.0%)	23 (92.0%)	23 (92.0%)	22 (88.0%)	22 (88.0%)	25 (100.0%)	23 (92.0%)	20 (80.0%)
	Inflammation, neutrophilic	bilateral			1 (4.0%)					
					1 (4.0%)					
heart	Degeneration/necrosis, myocardium	focal	1 (4.0%)							
			1 (4.0%)							
	Infiltrate, mononuclear cell	focal; multifocal	2 (8.0%)	1 (4.0%)		1 (4.0%)	3 (12.0%)	2 (8.0%)	1 (4.0%)	1 (4.0%)
			2 (8.0%)	1 (4.0%)		1 (4.0%)	3 (12.0%)	2 (8.0%)	1 (4.0%)	1 (4.0%)
gut-associated lymphoid tissue	Infiltrate, neutrophils	focal							1 (4.0%)	
									1 (4.0%)	
gland, thyroid	Thymus, ectopic	focal	1 (4.0%)	3 (12.0%)		3 (12.0%)	6 (24.0%)	7 (28.0%)	8 (32.0%)	5 (20.0%)
			1 (4.0%)	3 (12.0%)		3 (12.0%)	6 (24.0%)	7 (28.0%)	8 (32.0%)	5 (20.0%)
gland, seminal vesicle	Infiltrate, mixed cell	focal		1 (4.0%)						
				1 (4.0%)						
gland, salivary, submandibular	Atrophy	diffuse	1 (4.0%)							
			1 (4.0%)							
	Ectasia, duct	diffuse	1 (4.0%)							
			1 (4.0%)							
	Infiltrate, mononuclear cell	focal; multifocal	10 (40.0%)	5 (20.0%)	7 (28.0%)	12 (48.0%)	9 (36.0%)	9 (36.0%)	9 (37.5%)	9 (36.0%)
			10 (40.0%)	5 (20.0%)	7 (28.0%)	12 (48.0%)	9 (36.0%)	9 (36.0%)	9 (37.5%)	9 (36.0%)
gland, salivary, sublingual	Infiltrate, mixed cell	focal								1 (4.0%)
										1 (4.0%)

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
	Infiltrate, mononuclear cell	focal; multifocal	1 (4.2%)		1 (4.0%)					
			1 (4.2%)		1 (4.0%)					
gland, salivary, parotid	Atrophy	focal		1 (4.0%)						
				1 (4.0%)						
	Infiltrate, mixed cell	multifocal							1 (4.2%)	
									1 (4.2%)	
	Infiltrate, mononuclear cell	focal		1 (4.0%)				1 (4.0%)		
				1 (4.0%)				1 (4.0%)		
	Inflammation, vessel	focal					1 (4.0%)			
							1 (4.0%)			
gland, prostate	Hyperplasia	focal		1 (4.0%)						
				1 (4.0%)						
	Infiltrate, mononuclear cell	focal; multifocal	12 (48.0%)	8 (32.0%)	12 (48.0%)	7 (28.0%)				
		focal			1 (4.0%)					
			12 (48.0%)	8 (32.0%)	13 (52.0%)	7 (28.0%)				
gland, preputia	Cyst, epithelial	unilateral				1 (4.0%)				
						1 (4.0%)				
	Inflammation, mixed cell, chronic	unilateral				1 (4.0%)				
						1 (4.0%)				
						2 (8.0%)				
gland, mammary	Hyperplasia	focal; multifocal						1 (4.2%)	2 (8.0%)	
		multifocal					2 (8.0%)			
		focal; multifocal						1 (4.2%)		
							2 (8.0%)	2 (8.3%)	2 (8.0%)	
gland, lacrimal	Atrophy	focal								1 (4.0%)
		unilateral				1 (4.0%)				
						1 (4.0%)				1 (4.0%)
	Ectasia, duct	unilateral				1 (4.0%)				
						1 (4.0%)				
	Infiltrate, mixed cell	focal; multifocal					1 (4.3%)	2 (8.0%)	2 (8.3%)	3 (12.0%)

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
							1 (4.3%)	2 (8.0%)	2 (8.3%)	3 (12.0%)
	Infiltrate, mononuclear cell	focal; multifocal	4 (16.0%)	8 (32.0%)	2 (8.0%)	6 (24.0%)	8 (34.8%)	11 (44.0%)	7 (29.2%)	8 (32.0%)
			4 (16.0%)	8 (32.0%)	2 (8.0%)	6 (24.0%)	8 (34.8%)	11 (44.0%)	7 (29.2%)	8 (32.0%)
gland, harderian	Hyperplasia	focal	1 (4.0%)	1 (4.0%)	1 (4.0%)					
			1 (4.0%)	1 (4.0%)	1 (4.0%)					
	Infiltrate, mononuclear cell	focal; multifocal	4 (16.0%)	7 (28.0%)	5 (20.0%)	6 (25.0%)	7 (28.0%)	8 (32.0%)	6 (25.0%)	10 (40.0%)
		multifocal							1 (4.2%)	
			4 (16.0%)	7 (28.0%)	5 (20.0%)	6 (25.0%)	7 (28.0%)	8 (32.0%)	7 (29.2%)	10 (40.0%)
gland, clitoral	Infiltrate, mixed cell	focal; multifocal					4 (16.0%)	6 (25.0%)	4 (16.7%)	6 (25.0%)
							2 (8.0%)	2 (8.3%)		2 (8.3%)
							6 (24.0%)	8 (33.3%)	4 (16.7%)	8 (33.3%)
	Inflammation, vessel	focal							1 (4.2%)	
									1 (4.2%)	
gland, adrenal cortex	Hyperplasia, subcapsular cell	focal; multifocal	14 (56.0%)	16 (64.0%)	12 (48.0%)	10 (40.0%)	19 (76.0%)	22 (88.0%)	20 (83.3%)	16 (64.0%)
		multifocal					6 (24.0%)	1 (4.0%)	3 (12.5%)	8 (32.0%)
			14 (56.0%)	16 (64.0%)	12 (48.0%)	10 (40.0%)	25 (100.0%)	23 (92.0%)	23 (95.8%)	24 (96.0%)
	Hypertrophy	focal; zona fasciculata			1 (4.0%)					
					1 (4.0%)					
gallbladder	Hypertrophy, epithelium	focal; multifocal		1 (4.0%)	2 (8.3%)	2 (8.3%)	1 (4.2%)	1 (4.0%)		1 (4.0%)
				1 (4.0%)	2 (8.3%)	2 (8.3%)	1 (4.2%)	1 (4.0%)		1 (4.0%)
	Infiltrate, mixed cell	focal; focal; mucosa/submucosa; multifocal; mucosa/submucosa	1 (4.0%)	3 (12.0%)	3 (12.5%)	3 (12.5%)	2 (8.3%)	1 (4.0%)	1 (4.2%)	1 (4.0%)
			1 (4.0%)	3 (12.0%)	3 (12.5%)	3 (12.5%)	2 (8.3%)	1 (4.0%)	1 (4.2%)	1 (4.0%)
	Infiltrate, mononuclear cell	focal; focal; mucosa/submucosa; multifocal	4 (16.0%)		1 (4.2%)	1 (4.2%)	2 (8.3%)	4 (16.0%)	3 (12.5%)	3 (12.0%)
			4 (16.0%)		1 (4.2%)	1 (4.2%)	2 (8.3%)	4 (16.0%)	3 (12.5%)	3 (12.0%)
esophagus	Degeneration, muscularis	focal			3 (12.0%)	1 (4.0%)	2 (8.0%)			
					3 (12.0%)	1 (4.0%)	2 (8.0%)			

Organ/Tissue	Finding	Result modifier	Male (mg/kg/day)				Female (mg/kg/day)			
			Control	G2 - 0.5	G3 - 1	G4 - 2	Control	G2 - 0.5	G3 - 1	G4 - 3
	Infiltrate, mixed cell	focal; focal; submucosa; multifocal	1 (4.0%)		1 (4.0%)			2 (8.0%)	3 (12.0%)	
			1 (4.0%)		1 (4.0%)			2 (8.0%)	3 (12.0%)	
epididymis	Cell debris, luminal	bilateral; unilateral	2 (8.0%)	2 (8.0%)	1 (4.0%)	1 (4.0%)				
			1 (4.0%)	1 (4.0%)		2 (8.0%)				
			3 (12.0%)	3 (12.0%)	1 (4.0%)	3 (12.0%)				
	Granuloma, sperm	focal; unilateral	1 (4.0%)	1 (4.0%)						
			1 (4.0%)	1 (4.0%)						
	Hyperplasia	focal		1 (4.0%)						
				1 (4.0%)						
	Infiltrate, mixed cell	multifocal	1 (4.0%)		1 (4.0%)	1 (4.0%)				
			1 (4.0%)		1 (4.0%)	1 (4.0%)				
	Infiltrate, mononuclear cell	focal; multifocal	6 (24.0%)	4 (16.0%)	4 (16.0%)	1 (4.0%)				
			6 (24.0%)	4 (16.0%)	4 (16.0%)	1 (4.0%)				
	Reduced sperm, luminal	bilateral	1 (4.0%)	1 (4.0%)		1 (4.0%)				
		bilateral; unilateral	1 (4.0%)	2 (8.0%)		2 (8.0%)				
			2 (8.0%)	3 (12.0%)		3 (12.0%)				
brain	Infiltrate, mononuclear cell	multifocal							1 (4.0%)	
									1 (4.0%)	
	Inflammation, vessel	focal							1 (4.0%)	
									1 (4.0%)	
bone, sternum	Necrosis	focal; articular cartilage						2 (8.0%)	1 (4.0%)	
								2 (8.0%)	1 (4.0%)	

5 Pages have been Withheld in Full as b4 (CCI/TS) immediately following this page

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This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

GOWRA G JAGADEESH
09/22/2021 09:50:45 AM

XUAN CHI
09/22/2021 10:19:00 AM
Concur.